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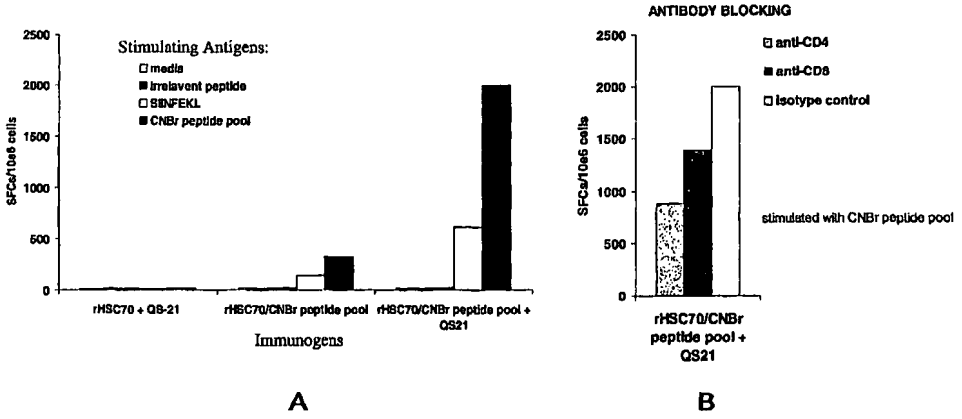

(57) Abstract: The present invention relates to methods for making complexes of HSP or alpha-2 macroglobulin and antigenic peptides, and uses of such complexes for the prevention and treatment of infectious diseases, cancers, and metabolic disorders. The methods of the invention comprises treating a purified target antigen preparation with a protease and/or a chemical to generate an antigenic set of peptides which is complexed to HSP or alpha-2 macroglobulin. Also encompassed in the invention are compositions comprising complexes of HSP or alpha-2 macroglobulin and antigenic peptides.

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METHODS FOR MAKING COMPOSITIONS COMPRISING HEAT SHOCK PROTEINS OR ALPHA-2-MACROGLOBULIN FOR THE TREATMENT OF CANCER AND INFECTIOUS DISEASE

[0001] This application claims the benefit of United States provisional patent application serial no. 60/574,510, filed May 25, 2004 and provisional application serial no. 60/588,902, filed July 16, 2004, each of which is incorporated by reference herein in its entirety.

[0002] This invention was made with government support under grant number 1R43 AI055185-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

1. INTRODUCTION

[0003] The present invention relates to methods and compositions for eliciting an immune response in a subject against a protein. The methods and compositions can also be used for the prevention and treatment of infectious diseases, primary and metastatic neoplastic diseases, and metabolic diseases. In the practice of the prevention and treatment of infectious diseases, cancer and metabolic diseases, compositions comprising peptides generated from a purified protein are complexed to heat shock proteins and/or alpha-2-macroglobulin to stimulate the immune response to metabolic target molecules, tumors, and infectious agents. The uses of such compositions in combination with other treatment modalities are also encompassed.

2. BACKGROUND OF THE INVENTION

2.1. HEAT SHOCK PROTEINS

[0004] Heat shock proteins (HSPs), also referred to as stress proteins, were first identified as proteins synthesized by cells in response to heat shock. HSPs have been classified into five families, based on molecular weight, HSP100, HSP90, HSP70, HSP60, and smHSP. Many members of these families were found subsequently to be induced in response to other stressful stimuli including nutrient deprivation, metabolic disruption, oxygen radicals, and infection with intracellular pathogens (see Welch, May 1993, Scientific American 56-64; Young, 1990, Annu. Rev. Immunol. 8:401-420; Craig, 1993, Science 260:1902-1903; Gething et al., 1992, Nature 355:33-45; and Lindquist et al., 1988, Annu. Rev. Genetics 22:631-677). These families also contain constituitively expressed homologs of the induced proteins.

[0005] Studies on the cellular response to heat shock and other physiological stresses revealed that the HSPs are involved not only in cellular protection against these adverse conditions, but also in essential biochemical and immunological processes in unstressed cells. HSPs accomplish different kinds of chaperoning functions. For example, members of the HSP70 family, located in the cell cytoplasm, nucleus, mitochondria, or endoplasmic reticulum (Lindquist et al., 1988, Ann. Rev. Genetics 22:631-677), are involved in the presentation of antigens to the cells of the immune system, and are also involved in the transfer, folding and assembly of proteins in normal cells. HSPs are capable of binding proteins or peptides, and releasing the bound proteins or peptides in the presence of adenosine triphosphate (ATP) or acidic conditions (Udono and Srivastava, 1993, J. Exp. Med. 178:1391-1396).

[0006] Srivastava et al. demonstrated immune response to methylcholanthrene-induced sarcomas of inbred mice (1988, Immunol. Today 9:78-83). In these studies, it was found that the molecules responsible for the individually distinct immunogenicity of these tumors were glycoproteins of 96kDa (gp96) and intracellular proteins of 84 to 86kDa (Srivastava et al., 1986, Proc. Natl. Acad. Sci. USA 83:3407-3411; Ullrich et al., 1986, Proc. Natl. Acad. Sci. USA 83:3121-3125). Immunization of mice with gp96 or p84/86 isolated from a particular tumor rendered the mice immune to that particular tumor, but not to antigenically distinct tumors. Isolation and characterization of genes encoding gp96 and p84/86 revealed significant homology between them, and showed that gp96 and p84/86 were, respectively, the endoplasmic reticular and cytosolic counterparts of the same heat shock proteins (Srivastava et al., 1988, Immunogenetics 28:205-207; Srivastava et al., 1991, Curr. Top. Microbiol. Immunol. 167:109-123). Further, HSP70 was shown to elicit immunity to the tumor from which it was isolated but not to antigenically distinct tumors. However, HSP70 depleted of peptides was found to lose its immunogenic activity (Udono and Srivastava, 1993, J. Exp. Med. 178:1391-1396). These observations suggested that the heat shock proteins are not immunogenic per se, but form noncovalent complexes with antigenic peptides, and the complexes can elicit specific immunity to the antigenic peptides (Srivastava, 1993, Adv. Cancer Res. 62:153-177; Udono et al., 1994, J. Immunol., 152:5398-5403; Suto et al., 1995, Science 269:1585-1588).

[0007] Noncovalent complexes of HSPs and peptide, purified from cancer cells, can be used for the treatment and prevention of cancer and have been described in PCT publications WO 96/10411, dated April 11, 1996, and WO 97/10001, dated March 20, 1997 (see also U.S. Patent No. 5,750,119 issued May 12, 1998, and U.S. Patent No. 5,837,251 issued November 17, 1998, respectively, each of which is incorporated by reference herein

in its entirety). The isolation and purification of HSP-peptide complexes has been described, for example, from pathogen-infected cells, and used for the treatment and prevention of infection caused by the pathogen, such as viruses, and other intracellular pathogens, including bacteria, protozoa, fungi and parasites (see, for example, PCT Publication WO 95/24923, dated September 21, 1995). Immunogenic stress protein-antigen complexes can also be prepared by in vitro complexing of stress protein and an antigenic protein, and the uses of such complexes for the treatment and prevention of cancer and infectious diseases has been described in PCT publication WO 97/10000, dated March 20, 1997 (U.S. Patent No. 6,030,618 issued February 29, 2000). The use of stress protein-antigen complexes for sensitizing antigen presenting cells in vitro for use in adoptive immunotherapy is described in PCT publication WO 97/10002, dated March 20, 1997 (see also U.S. Patent No. 5,985,270 issued November 16, 1999).

2.2. ALPHA-2-MACROGLOBULIN

[0008] The α-macroglobulins are members of a protein superfamily of structurally related proteins which also comprises complement components C3, C4 and C5. The human plasma protein alpha-2-macroglobulin (α2M) is a 720 kDa homotetrameric protein primarily known as proteinase inhibitor and plasma and inflammatory fluid proteinase scavenger molecule (for review see Chu and Pizzo, 1994, Lab. Invest. 71:792). α2M is synthesized as a precursor having 1474 amino acid residues. The first 23 amino acids function as a signal sequence that is cleaved to yield a mature protein with 1451 amino acid residues (Kan et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82:2282-2286).

[0009] α2M promiscuously binds to proteins and peptides with nucleophilic amino acid side chains in a covalent manner (Chu et al., 1994, Ann. N.Y. Acad. Sci. 737:291-307) and targets them to cells which express a α2M receptor (α2MR) (Chu and Pizzo, 1993, J. Immunol. 150:48). Binding of α2M to the α2M receptor is mediated by the carboxy-terminal portion of α2M (Holtet et al., 1994, FEBS Lett. 344:242-246) and key residues have been identified (Nielsen et al., 1996, J. Biol. Chem. 271:12909-12912).

[0010] Generally known for inhibiting protease activity, α2M binds to a variety of proteases through multiple binding sites (see, e.g., Hall et al., 1981, Biochem. Biophys. Res. Commun. 100(1):8-16). Protease interaction with α2M results in a complex structural rearrangement called transformation, which is the result of a cleavage within the "bait" region of α2M after the proteinase becomes "trapped" by thioesters. The conformational change exposes residues required for receptor binding, allowing the α2M-proteinase complex to bind to the α2MR. Methylamine can induce similar conformational changes and cleavage as that induced by proteinases. The uncleaved form of α2M, which is not

recognized by the receptor, is often referred to as the "slow" form (s-α2M). The cleaved form is referred to as the "fast" form (f-α2M) (reviewed by Chu et al., 1994, Ann. N.Y. Acad. Sci. 737:291-307). Recently, it has also been shown that the α2MR can bind to HSPs, such as gp96, hsp90, hsp70, and calreticulin (Basu et al., 2001, Immunity 14(3):303-13).

[0011] Studies have shown that in addition to its proteinase-inhibitory functions, α2M, when complexed to antigens, can enhance the antigens' ability to be taken up by antigen presenting cells such as macrophages and presented to T cell hybridomas in vitro by up to two orders of magnitude (Chu and Pizzo, 1994, Lab. Invest. 71:792; WO 99/50303; US 6403092 and WO94/14976), and to induce T cell proliferation (Osada et al., 1987, Biochem. Biophys. Res. Commun.146:26-31). Further evidence suggests that complexing antigen with α2M enhances antibody production by crude spleen cells in vitro (Osada et al., 1988, Biochem. Biophys. Res. Commun. 150:883), elicits an in vivo antibody response in experimental rabbits (Chu et al., 1994, J. Immunol. 152:1538-1545) and mice (Mitsuda et al., 1993, Biochem. Biophys. Res. Commun. 101:1326-1331). α2M-antigenic peptide complexes have also been shown to induce a cytotoxic T cell response in vivo (Binder et al., 2001, J. Immunol. 166:4698-49720).

3. SUMMARY OF THE INVENTION

[0012] The present invention encompasses the making of complexes comprising antigenic peptides and heat shock protein (HSP) or alpha-2-macroglobulin (α2M) and using such complexes for eliciting an immune response and the prevention and treatment of cancer and infectious disease.

[0013] In one embodiment, the invention provides a method of making an immunogenic population of complexes comprising heat shock protein or alpha-2-macroglobulin, wherein a purified target antigen preparation or combination of different purified target antigen preparations is treated with a protease and/or a non-enzymatic chemical agent that cleaves polypeptides to generate an antigenic set of peptides, and complexing the antigenic set of peptides to heat shock protein or alpha-2-macroglobulin to form the population of complexes. Optionally, after the complexing step, the complexes are purified. Each of the different purified target antigen preparations comprises a purified polypeptide target antigen. Also encompassed are compositions comprising complexes of HSP or α2M and antigenic peptides prepared by the methods taught herein, and pharmaceutical compositions comprising such complexes. The complexes are preferably purified in such compositions.

[0014] In another embodiment, the invention provides a method of eliciting an immune response in a subject to one or more target antigens. The subject is administered with a population of complexes produced by a method wherein a purified target antigen preparation or combination of different purified target antigen preparations is treated with a protease and/or a compound that cleaves polypeptide to generate an antigenic set of peptides, each of the different purified target antigen preparations comprising a purified polypeptide target antigen; and the antigenic set of peptides is complexed to heat shock protein or alpha-2-macroglobulin to form the population of complexes. Preferably, the elicited immune response is a type-1 response mediated by Th1-type T cells and/or a type-2 response mediated by Th2-type T cells. In certain embodiments, the immune response is characterized by activation, preferably antigen-specific activation, of cytotoxic T cells, NK cells, and/or B cells.

[0015] In yet another embodiment, the invention provides a method of treating or preventing a type of cancer in a subject. The subject is administered with a population of complexes produced by a method wherein a purified target antigen preparation or combination of different purified target antigen preparations is treated with a protease and/or a compound that cleaves polypeptide to generate an antigenic set of peptides; and the antigenic set of peptides is complexed to heat shock protein or alpha-2-macroglobulin to form the population of complexes. Each of the different purified target antigen preparations comprises a purified polypeptide target antigen. In this context, the target antigen can be a unique tumor antigen, a shared tumor specific antigen, a shared tumor associated antigen, a differentiation antigen, or an antigen overexpressed in cells or tissue of said type of cancer.

[0016] In yet another embodiment, the invention provides a method of treating or preventing a type of infectious disease. The subject is administered with a population of complexes produced by a method wherein a purified target antigen preparation or combination of different purified target antigen preparations is treated with a protease and/or a compound that cleaves polypeptide to generate a antigenic set of peptides; and the antigenic set of peptides is complexed to heat shock protein or alpha-2-macroglobulin to form the population of complexes. Each of the different purified target antigen preparations comprises a purified polypeptide target antigen. In certain embodiments, the target antigen in this context is an antigen that is present in a cell when infected with a pathogen that causes the infectious disease, that is of an pathogen that causes the infectious disease, or that comprises an antigenic determinant of the pathogen.

[0017] In yet another embodiment, the invention provides a method of treating and preventing a variety of metabolic disorders in which proteins that can be targeted for drug

action are identified, such as but not limited to, cardiovascular disorders, neurological disorders, and hormonal disorders. In certain embodiments, the compositions of the invention are administered in combination with one or more other therapeutic modalities to treat or prevent such a disorder.

[0018] In certain embodiments, a recombinant or synthetic target antigen that comprises at least one antigenic determinant of one or more different target antigens, can be used. Such chimeric target antigens may optionally be separated by linker sequences that comprise cleavage sites.

[0019] In various embodiments, the subject in which a disease or disorder is to be treated or prevented, or an immune response is elicited, can be a non-human vertebrate, a mammal, or a human.

[0020] In various embodiments, the target antigen can be derived from cancer cells, or cells infected with a pathogen or infectious agent, and preferably derived from human cells, or can be, or can be derived from, purified or recombinant proteins. The target antigen can also be derived from cells of a pathogen or infectious agent, or variants thereof. The target antigen can be prepared from cancer cells or cells infected with a pathogen that are antigenically related to the cancer or the pathogen that causes infectious diseases. A pathogen or infectious agent, or a non-infectious form of the infectious agent, including viral particles and bacterial cells, can also be used as a source of the target antigen. In a specific embodiment, the target antigen can be made by lysing one or more antigenic cells, removing cell debris and non-proteinaceous materials, and purifying the target antigen by methods known in the art. In various embodiments, different purified target antigen preparations are combined before treatment with protease or non-enzymatic chemical cleavage agent.

[0021] In certain embodiments, the target antigen can be digested by one or more of a variety of proteases (including but not limited to a purified protease, a protein complex that shows protease activity, or a proteosome) or non-enzymatic chemicals, such as but not limited to trypsin, Staphylococcal peptidase I (also known as protease V8), chymotrypsin, pepsin, cathepsin G, thermolysin, elastase, papain, and cynaogen bromide, under conditions suitable for the reaction. Preferably, the enzyme(s) and/or non-enzymatic chemical(s) selected to cleave a protein are such that the cleavage site specificity of the enzymes and/or non-enzymatic chemicals does not cleave a known epitope of the protein. The extent of the digestion can be monitored by taking a sample and analyzing it by known techniques for determining the length of peptides.

[0022] In one embodiment, the digesting step is carried out under conditions such that the resulting population of peptides which preferably comprises antigenic peptides, have an average size of from about 7 amino acid residues to about 20 amino acid residues. In another embodiment, the resulting population of peptides is subjected to a separation procedure (e.g., ultrafiltration, column chromatography) and the peptides of less than 10 KDa are recovered for use. A target antigen can be subjected to cleavage by more than one proteases and/or non-enzymatic chemicals, or a combination of proteases and non-enzymatic chemicals, sequentially or simultaneously. It is also desirable to generate from a target antigen different antigenic sets of peptides by digesting aliquots of the target antigen with different proteases and/or chemicals. The peptides resulting from the different digests may be combined before complexing to HSP or α2M. Before complexing the antigenic set of peptides to HSP or α2M, it may be desirable to inactivate or separate the protease and/or non-enzymatic chemical cleavage agent from the peptides, and optionally to purify the set of antigenic peptides.

[0023] In various embodiments of the invention, depending on the method used to complex the antigenic set of peptides to HSP or α2M in vitro, the reaction can result in the antigenic set of peptides complexed to HSP or α2M by either a covalent bond or non-covalent bond. Heat shock proteins that are contemplated for complexing include but are not limited to HSP 60, HSP 70, HSC 70, HSP 90, gp96, calreticulin, grp78 (or BiP), protein disulfide isomerase (PDI), HSP110, and grp170. It is generally preferred to use HSP or α2M from the same species to which the complexes will be administered. Human HSPs and human α2M are preferred. The complexes of HSP or α2M and antigenic set of peptides formed in vitro can optionally be further purified before their use in or as a therapeutic or prophylactic composition. Such compositions of the invention may comprise a pharmaceutically acceptable carrier, and may further comprise an adjuvant. Kits comprising HSP and/or α2M, antigenic sets of peptides, and/or proteases, and additional treatment modalities are also provided.

[0024] In another aspect, a method is provided for treating or preventing a type of cancer or infectious disease, comprising administering to a subject in need of such treatment or prevention (i) a composition comprising an amount, effective for said treatment or prevention, of HSP and/or α2M complexed to antigenic peptides; and optinally in combination with (ii) another treatment modality that is a non-HSP and non-α2M-based treatment modality. The additional treatment modality can be a non-vaccine treatment modality. Examples of treatment modalities include but are not limited to antibiotics, antivirals, antifungal compounds, antiprotozoal compounds, antihelminth compounds, anti-

cancer treatments such as chemotherapeutic agents, antiangiogenic compounds, hormones, and radiation, as well as drugs, biological therapeutic agents and immunotherapeutic agents.

[0025] In another embodiment, a method is provided for treating or preventing a type of cancer or infectious disease, comprising administering to a subject in need of such treatment or prevention antigen presenting cells which have been sensitized with complexes of HSP and/or α2M and antigenic peptides made according to the invention. In addition to the administration of sensitized antigen presenting cells to a subject, complexes of HSP and/or α2M and antigenic peptides; and/or a non-HSP and non-α2M-based treatment modality can also be administered to the subject.

[0026] In yet another embodiment of the invention, a method is provided for eliciting an immune response in a subject against a first target antigen, wherein said subject is receiving a non-HSP and non-α2M treatment modality, said method comprising administering to the individual a composition comprising an immunogenic amount of HSP and/or α2M complexed to an antigenic set of peptides that were prepared from a second target antigen. The antigenic set of peptides can be obtained by digesting the second target antigen with a protease. The first and second target antigens express at least one common antigenic determinant.

[0027] The administering of the HSP complexes or α2M complexes to a subject can be repeated at the same site or different sites, and periodically, for example, at weekly intervals. The composition can be administered by many routes, such as intradermally or subcutaneously. The HSP complexes or α2M complexes can be administered to a subject in combination with an adjuvant, such as but not limited to QS21. The HSP/α2M complexes can be administered over a period of time which may precede, overlap, and/or follow a treatment regimen with a non-vaccine treatment modality.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0028] Figs. 1A and 1B. OVA CNBr peptides complexed to rh-HSC70 induce SIINFEKL- and CNBr-cleaved peptide-specific responses. Fig. 1A Graph of immune response to stimulating antigen in spleen cells from C57BL/6 mice immunized with 1) 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant, 2) 100 μg of rh-HSC70 complexed with OVA CNBr-generated peptide set, or 3) 100 μg of rh-HSC70 complexed with OVA CNBr-generated peptide set plus 10 μg per injection of QS-21 adjuvant. On day 14, the splenocytes were isolated and seted splenocytes from 3 mice were subjected to IFN-γ ELISPOT analysis. Y axis shows numbers of IFN-γ SFCs per 1e6 splenocytes after in vitro re-stimulation with 10 μg/ml of peptide or peptide set as indicated for 40 hr. Fig. 1B.

Splenocytes of C57BL/6 mice immunized with rh-HSC70 complexed with OVA CNBr-generated peptide set plus 10 μg per injection of QS-21 adjuvant show CD4- and CD8-specific immune responses to the CNBr-generated peptide set. C57BL/6 mice were intradermally immunized on day 0 and 7 with 100 μg rh-HSC70/CNBr peptides complex (at 1:5 molar ratio of rh-HSC70 to peptides), equivalent amount of CNBr peptides, or rHSC70 alone, with 10 μg per injection of QS-21. On day 14, the splenocytes were isolated and pooled splenocytes from 3 mice were subjected to IFN-γ ELISPOT analysis. Y axis shows numbers of IFN-γ SFCs per 1e6 splenocytes. Tested splenocytes were pre-incubated with anti-CD4, anti-CD8 or isotype control antibody (at 10 μg/ml final concentration) and re-stimulated with 10 μg/ml of CNBr peptide set for 40 hr.

[0029] Fig. 2A-2D. Mixture of OVA CNBr- and V8 protease- cleavage-generated peptides complexed to rh-HSC70 induces stronger SIINFEKL- and CNBr /V8 peptide-specific responses than OVA CNBr- or V8 protease- cleavage-generated peptides alone complexed to rh-HSC70. C57BL/6 mice were intradermally immunized on day 0 with 100 μg rh-HSC70/CNBr peptides complex (at 1:5 molar ratio of rh-HSC70 to peptides), equivalent amount of CNBr peptides, or rHSC70 alone, with 10 μg per injection of QS-21. On day 8, the splenocytes were isolated and pooled splenocytes from 3 mice were subjected to IFN-γ ELISPOT analysis. Y axis shows numbers of IFN-γ SFCs per 1e6 splenocytes after in vitro re-stimulation with 10 μg/ml of peptide or peptide set as indicated for 40 hr. Fig. 2A, Graph of immune response to stimulating antigen in spleen cells from mice immunized with 1) 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant, 2) 10.71 μg of OVA CNBr- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant, or 3) 100 μg of rh-HSC70 complexed with OVA CNBr-cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant. Fig. 2B, Graph of immune response to peptide set as stimulating antigen in spleen cells from mice immunized with 1) 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant, 2) 10.71 μg of OVA V8 protease- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant, or 3) 100 μg of rh-HSC70 complexed with OVA V8 protease- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant. Fig. 2C. Graph of immune response to stimulating antigen in spleen cells from mice immunized with 1) 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant, 2) 21.42 μg of OVA CNBr- and V8 protease- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant, or 3) 100 μg of rh-HSC70 complexed with a mixture of OVA CNBr- and V8 protease- cleavage-generated peptide sets plus 10 μg per injection of QS-21 adjuvant. Fig. 2D. Mixture of OVA CNBr and V8 peptides complexed to rh-HSC70 induced CD8 responses, which could be blocked by anti-CD8 antibody. Graph of

immune response to stimulating antigen in spleen cells from mice immunized with 100 μg of rh-HSC70 complexed with OVA CNBr- and V8 protease- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant.

[0030] Fig. 3A and 3B. Prophylactic and therapeutic efficiacies of CNBr and V8 peptides complexed to mHSP70 in a mouse model of tumor. Fig. 3A. Histogram of the mean tumor volume on day 22 of five groups of mice immunized respectively with PBS, 100 μg of mHSP70 with 10 μg of QS-21, 100 μg of OVA CNBr and V8 peptides with 10 μg of QS-21, 100 μg mHsp 70 complexed with OVA CNBr and V8 peptides and 10 μg of QS-21 and 25 μg of OVA with 10 μg of QS-21, and then challenged by injection of EG7-OVA cells. The data demonstrated the prophylactic use of antigenic peptides complexed to hsp in preventing growth of tumor cells in animals. Fig. 3B. Histogram of the mean tumor volume on day 22 post-tumor inoculation in five groups of mice treated respectively with PBS, 100 μg of mHSP70 with 10 μg of QS-21, 100 μg of OVA CNBr and V8 peptides with 10 μg of QS-21, 100 μg of mHSP70 complexed with OVA CNBr and V8 peptides and 10 μg of QS-21 and 25 μg of OVA with 10 μg of QS-21. The data demonstrated the therapeutic use of antigenic peptides complexed to hsp in treating animals with pre-existing tumor.

5. DETAILED DESCRIPTION OF THE INVENTION

[0031] The present invention provides methods for preparing and using a composition comprising heat shock protein (HSP) or alpha-2-macroglobulin (α2M) useful for the prevention or treatment of cancer and infectious disease. The methods of the invention are useful for designing vaccines against cancer cells or infectious agents, and comprise complexing HSP to antigenic peptides in vitro. In particular, the methods comprise using a purified protein as a source of immunogens. The invention further comprises various methods to generate a broad spectrum of peptides from the protein which are complexed to HSP for presentation to T cells. Also encompassed are compositions comprising complexes of HSP or α2M and antigenic peptides prepared by the methods taught herein, and pharmaceutical compositions comprising such complexes. The complexes are preferably purified in such compositions.

[0032] Selection of appropriate peptides is generally a critical step in any effort to elicit or enhance peptide-specific protective cellular immunity. Information regarding such immunogenic peptides for a particular pathogen is not generally available. One advantage of the present invention is to provide a means to test and optimize a set of immunogenic peptides which can be used in a vaccine.

[0033] The compositions and methods of the present invention are an improvement over other compositions and methods that use naturally-occurring HSP-antigenic peptide complexes to treat or prevent cancer or infectious disease. In such methods, a specific HSP and its complexes with antigenic peptides are isolated from a cancer or infected cell, and administered to a patient to induce an immune response against the cancer or infected cells in vivo (see e.g., U.S. Patent Nos. 5,750,119 and 5,961,979). Naturally-occurring complexes are isolated by methods dictated by the type of HSP which is desired. Thus, naturally-occurring complexes of a type of HSP and antigenic peptides comprise only those antigenic peptides that are co-localized in a compartment of the antigenic cells with that type of HSP. Certain types of HSPs are found uniquely in one cellular compartment and some antigenic peptides are found only in certain compartments of an antigenic cell. The methods of the present invention complex sets of antigenic peptides to one or more different HSP which can then be used to stimulate an immune response in a subject. By using the methods of the invention, even antigenic peptides and HSPs that are not co-localized can form a complex. The methods of the invention afford the possibility to form complexes of a particular type of HSP with peptides of any desired protein.

[0034] The composition of peptides associated with naturally-occuring HSP complexes is dictated in part by the type of proteases present in the various cellular compartments that an antigenic protein moves through in a cell. Given a particular protein, the cellular proteases may generate in vivo a limited number of peptides of different antigenicities. However, by making use of the ability to use proteases with specificities different from those cellular enzymes and chemical agents in the methods of the invention, a greater number of different peptides can be created in vitro from the specific protein for complexing to HSPs. As a result, the diversity of peptides of different antigenicities and/or immunogenicities from a particular protein that can be presented to the immune system is increased.

[0035] The HSP-peptide complexes and α2M-peptide complexes in the compositions of the invention provide an extensive display of potential antigenic regions of the target protein or polypeptide of interest, which can be used to stimulate an immune response to as many distinct regions or epitopes of the antigenic protein as possible. A strong and long-lasting immune response in a subject to a target antigen based on multiple diverse epitopes is thus expected to be achieved. In one embodiment, the immune response is a cell-mediated immune response which enables T8-lymphocytes to proliferate and differentiate into cytotoxic T cells capable of destroying infected host cells, mutant cells, or cancer cells, activate cytotoxic T cells and NK cells, promote the proliferation of T4-

lymphocytes, activate macrophages to destroy intracellular pathogens, stimulate the production of opsonizing and complement-activating antibodies for enhanced attachment during phagocytosis, activate neutrophils, stimulate increased production of monocytes in the bone marrow, and allow for activation of adhesion molecules during diapedesis. A set of cytokines are produced during cell-mediated immune response, which includes interferon-gamma (IFN-gamma), interleukin-2(IL-2), interleukin-18(IL-18), interleukin-23(IL-23), interleukin-12(IL-12), interleukin-27 (IL-27), lymphotoxin, and tumor necrosis factor-alpha (TNF-alpha). The presence of an immune response can be assessed by measuring the levels of one or more of the type-1 cytokines, such as IFN-gamma, by assays well known in the art, e.g., ELISPOT as taught in Section 6.1.7 below. See Immunobiology by Charles Janeway et al., 6th edition, 2005, Garland Publishing, Chapter 10, which is incorporated herein by reference in its entirety. An increase in the production of IFN-gamma or activation of cytotoxic T cells and/or NK cells in response to a specific antigen indicates the presence of an immune response against the antigen. In a specific embodiment, the immune response is mediated by Th1-type T cells is elicited by the methods of the invention.

[0036] In another embodiment, a humoral immune response is elicited by the methods of the invention. A humoral response is characterized by activation of B cells to proliferate, stimulatation of activated B cells to synthesize and secrete antibodies, differentiation of B cells into antibody-secreting plasma cells, antibody class switching (e.g., IgG), activation of eosinophils and production of increased amounts of IgE which is particularly effective against helminths and arthropods. A set of cytokines are produced during a humoral immune response which includes interleukins 4, 5, 9, 10, and 13 (IL-4, IL-5, IL-9, IL-10, and IL-13) The presence of a humoral immune response can be detected by determining the cytokine profile, Ig classes of antibodies produced, and status of activations of various T cell subtypes. In a specific embodiment, the immune response is mediated by Th2-type T cells is elicited by the methods of the invention.

[0037] In certain embodiments, the immune response that is elicited by the methods and compositions of the invention is not an immune tolerance reaction, a desensitization to an antigen, inhibition of a pre-existing immune response, modification of the immune response of the subject towards a pathology associated with an allergic or autoimmune reaction or toward graft rejection phenomena such that the immune response of said subject comes close to the natural tolerance manifested by normal subjects to the target antigen. In many instances, immune tolerance is induced by antigens that reach the gut, such as dietary proteins, and allergic antigens in food. Immune tolerance is associated with secretion of

transforming growth factor beta (TGF-beta) by a certain subset of T cells, e.g. Th3 cells, which provides help for cells to switch from IgE and IgG antibody production to IgA, and has suppressive properties for both Th1 and Th2 cells in an non-antigen specific fashion.

[0038] The terms "elicit", "stimulate", and "induce" are used interchangeably to denote the generation of a de novo immune response in a subject or the increasing of the strength or persistence of an existing immune response.

[0039] Preferably, the immune response elicited by the compositions and methods of the invention is a type-1 immune response, or an immune response mediated by Th1 cells or primarily by Th1-type T cells. In another embodiment, a Th2 immune response or or an immune response mediated by Th2-type T cells or primarily by Th2-type T cells is elicted. In yet another embodiment, an immune response involving antigen-specific activation of Th1- and Th2-type T cells is elicited. In yet another embodiment, an immune response that is not associated with activity of Th3-type T cells and/or antigen-specific activation of Th3-type T cells is elicited.

[0040] As used herein, the term "target antigen" refers to a protein or polypeptide to which an immune response in a subject is desired. The terms "protein" and "polypeptide" are used herein interchangeably. The term "antigenic" as used herein describes a molecule to which an antibody binds. The term "immunogenic" denotes a molecule that is capable of eliciting or stimulating an immune response to itself in a subject. Section 5.1 describes target antigens and methods of preparation and purification from various sources. According to the invention, a set of peptides is generated from the target antigen by various methods including enzymatic digestion and/or non-enzymatic chemical cleavage. Section 5.2 describes the methods used to generate antigenic sets of peptides from the target antigen. Section 5.3 describes the preparation of the heat shock protein or α2M in the complexes. The methods for making the complexes, the compositions, and their various pharmaceutical uses are described in Sections 5.4 to 5.7. Methods for comparing the immunogenicities of HSP- or α2M- peptide complexes using different antigenic sets of peptides is described in Section 5.8.

5.1. TARGET ANTIGENS

[0041] In various embodiments of the invention, the choice of target antigen depends on the nature of the disease, and the antigens that are associated with the disease. One or more target antigens (e.g., 2, 3, 4, or 5 different target antigens) can be employed in the methods of the invention either alone or in combination. For simplicity of explanation and without any limitation, a single target antigen is used in the description of the methods of the invention.

[0042] For the treatment or prevention of cancer, the methods of the invention provide compositions of HSPs and/or α2M complexed to antigenic peptides, which antigenic peptides were produced by various methods from a purified preparation of a target antigen associated with a cancer, or from a purified preparation of a molecule displaying the antigencity of a target antigen associated with a cancer. The target antigen can be a protein or polypeptide present in the cells and tissues of the cancer, preferably human cancers, for example, but not limited to, tumor-specific markers or antigens, and tumor-associated markers or antigens. HSP and/or α2M complexes comprising antigenic peptides derived from a target antigen of a cancer can generally be used to elicit an immune response to cells or tissue of the same type of cancer. As used herein, the term "cells or tissue of the same type of cancer" refers to cells or tissue of cancer of the same tissue type, or metastasized from cancer of the same tissue type.

[0043] In the context of treatment or prevention of cancer, a tumor antigen used in the methods of the invention can be a unique antigen, or a shared antigen. Many proteins are considered to be a tumor antigen based on their recognition by T lymphocytes that also recognize tumor cells expressing the protein. In various embodiments of the invention, the tumor antigens that can be used as target antigens can be divided into four groups. Unique antigens result from point mutations in genes. The mutation usually affects the coding region of the gene and is unique to the tumor of an individual patient or restricted to very few patients. Some of these mutations may be implicated in tumoral transformation. Such antigens, which are strictly tumor-specific, can play an important role in the natural anti-tumor immune response of individual patients, but they are not commonly shared by tumors from different patients.

[0044] Target antigens of the invention encompass shared antigens that are present on many independent tumors. One group corresponds to peptides encoded by "cancer-germline" genes, such as MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-5, MAGE-6, MAGE-7, MAGE-8, MAGE-9, MAGE-10, MAGE-11 and MAGE-12, which are expressed in many tumors but not in normal tissues. The only normal cells in which significant expression of such genes has been detected are placental trophoblasts and testicular germ cells. Because these cells do not express MHC class I molecules, gene expression does not appear to result in expression of the antigenic peptides and such antigens can therefore be considered as strictly tumor-specific. Other examples of shared tumor-specific antigens include but is not limited to BAGE-1, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, LAGE-1, LAGE-2, and SSX-2.

[0045] A second group of shared tumor antigens, named differentiation antigens, are also expressed in the normal tissue of origin of the malignancy. Non-limiting examples of differentiation antigens include but is not limited to tyrosinase, which is expressed in normal melanocytes and in most melanomas; carcinoembryonic antigen (CEA), an oncofetal protein expressed in normal colon epithelium and in most gut carcinomas, gp100/Pmel17, kallikrein 4, mammaglobin A, Melan-A, TRP-1, TRP-2 and prostate specific antigen (PSA).

[0046] The third group of shared antigens encompasses antigens which are expressed in a wide variety of normal tissues and are overexpressed in tumors. Examples of overexpressed antigens include but is not limited to Her-2/neu, CPSF, EphA3, alphafetoprotein, WT-1, telomerase, MUC-1, p53, PRAME, RAGE-1 and PSMA. The second and third groups of shared antigens are also known collectively as tumor associated antigens (TAAs).

[0047] Many cellular proteins that fit the above descriptions of expression patterns are known in the art as tumor antigens, or biomarkers associated with precancerous growth, cancer or metastasis, and are contemplated for use in the invention. Target antigens of the invention can be identified by cell biology methods, immunological methods, serological methods, as well as expression profiling using recombinant DNA and proteomics techniques such as differential display, SAGE, microarrays, and 2-D gel electrophoresis. For further descriptions, see Tumor Markers & Tumor Associated Antigens by Bimal C. Ghosh , Luna Ghosh, by McGraw-Hill (March 1987); Van den Eynde BJ, van der Bruggen P. T cell-defined tumor antigens. Curr Opin Immunol 1997 9: 684-93; Houghton AN, Gold JS, Blachere NE. Immunity against cancer: lessons learned from melanoma. Curr Opin Immunol 2001 13: 134-140; and van der Bruggen P, Zhang Y, Chaux P, Stroobant V, Panichelli C, Schultz ES, Chapiro J, Van den Eynde BJ, Brasseur F, Boon T. Tumor-specific shared antigenic peptides recognized by human T cells. Immunol Rev 2002; 188: 51-64; which are incorporated herein by reference in their entireties.

[0048] In certain embodiments, a target antigen of the invention is not an immunogenic or antigenic macromolecular structure that can induce graft rejection, allergic reaction, or autoimmune reaction in a human, such as the major histocompatibility complex I and II (MHC I and II), the minor histocompatibility antigens, alloantigens, xenoantigens, blood group antigens, plant allergens, pollen allergens, house dust mite allergens, food allergens, animal hair allergens, venom allergens, and mold allergens. In particular embodiments, a target antigen of the invention is not bovine beta-lactoglobulin, insulin, thyroglobulin, type II collagen, gliadin, GAD65, proteolipid protein, S-antigen, acetylcholin

receptor, napsenized colonic proteins, interphotoreceptor retinoid binding protein, myelin, peripheral nerve P2, LDL, HDL, phospholipase A2 from bee venom, MBP, Alt a2, or Bet 1a.

[0049] For the treatment or prevention of an infectious disease, the methods of the invention provide compositions of HSPs and/or α2M complexed to antigenic peptides, which antigenic peptides are produced by various methods from a purified preparation of a target antigen associated with the infectious disease. The target antigen can be a protein or polypeptide present in the pathogen or infectious agent which includes but is not limited to, a virus, bacterium, fungus, protozoan, helminth, multicellular parasite, and the like. The target antigen can also be a protein or polypeptide of a subject or host which is infected by a pathogen or infectious agent, wherein the protein or polypeptide is not normally present in host cells at a level that is associated with the infection. In one embodiment, the target antigen is a host protein that is induced or upregulated when the host cell is infected by the pathogen. Preferably, the pathogen is one that infects humans.

[0050] In various embodiments, surface antigens, surface glycoproteins, surface-exposed proteins, major surface antigens, minor surface antigens, capsid proteins, nucleocapsid proteins, serotype antigens, shared serotype proteins, serotype-specific proteins, haemagglutinins, neuramidases, coat proteins, mosaic proteins, spike proteins, matrix proteins, core proteins, envelope proteins, membrane-associated antigens, transmemberane proteins, lipidated proteins, virus attachment proteins, glycosylphosphatidylinositol-anchored proteins, extracellular proteins, docking proteins, secreted proteins, proteases, surface proteases, adhesins, porins, flagellins, adhesion proteins, internal antigens, intracellular antigens, major internal antigens, minor internal antigens, virulence factors, stress proteins, toxins, topoisomerases, gyrases, DNA polymerases, RNA polymerases, and reverse transcriptases of a pathogen can be target antigens of the invention.

[0051] Antigens of pathogens useful as target antigens for making the complexes of the invention can be identified by techniques commonly known in the art, such as but not limited to microbiological assays, immunological assays, serological assays, or bioinformatics. Antigens in use or under testing for use in a vaccine, such as a subunit vaccine, can be used as a target antigen of the invention. Examples of such antigens are described in The Jordan Report 2000, Accelerated Development of Vaccines, National Institute of Health, which is incorporated herein by reference in its entirety. Many antigens that can be used as target antigens of the invention for treatment of infectious diseases of non-human vertebrates are disclosed in Bennett, K. Compendium of Veterinary Products, 3rd ed. North American Compendiums, Inc., 1995, which is incorporated herein by

reference in its entirety. In certain embodiments, a target antigen of the invention is not, an antigen, a protein, a macromolecular recombinant protein or a mutated protein of a pathogen, such as virus, bacteria, fungi, protozoa, or helminth, that is responsible for infectious disease or parasitic diseases in humans.

[0052] As the genome of many pathogens are being sequenced, many antigens of these pathogens can be identified by examining the nucleotide sequences and the predicted amino acid sequences. Antigens identified by bioinformatics methods can also be used as target antigens of the invention. Non-limiting examples of such target antigens are described in Pizza, M. et al (2000), Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing. Science 287, 1816-1820; Grifantini, R. et al. (2002) Previously unrecognized vaccine candidates against group B meningococcus identified by DNA microarrays. Nat. Biotechnol. 20, 914-921; Ariel, N. et al. (2002) Search for potential vaccine candidate open reading frames in the Bacillus anthracis virulence plasmid pXO1: in silico and in vitro screening. Infect. Immun. 70, 6817-6827; Wizemann, T.M. et al. (2001) Use of a whole genome approach to identify vaccine molecules affording protection against Streptococcus pneumoniae infection. Infect. Immun. 69, 1593-1598; Etz, H. et al. (2002) Identification of in vivo expressed vaccine candidate antigens from Staphylococcus aureaus. Proc. Natl. Acad. Sci. U.S.A. 99, 6573-6578; Vytvytska, O. et al (2002) Identification of vaccine candidate antigens of Staphylococcus aureus by serological proteome analysis. Proteomics 2, 580-590; Montigiani, S. et al. (2002) Genomic approach for analysis of surface proteins in Chlamydia pneumoniae. Infect. Immun. 70, 368-379; Ross, B.C. et al. (2001) Identification of vaccine candidate antigens from a genomic analysis of Porphyromonas gingivalis. Vaccine 19, 4135-4142; Betts, J.C. (2002) Transcriptomics and proteomics: tools for the identification of novel drug targets and vaccine candidates for tuberculosis. IUBMB Life 53, 239-242, each of which is incorporated herein by reference in its entirety

[0053] Many viral proteins are known to be antigenic in animals including humans, and are contemplated for use in the invention as target antigens. In various embodiments, antigens of infectious virus of both human and non-human vertebrates, including retroviruses, RNA viruses and DNA viruses, can be used as target antigens. A target antigen of the invention can be an antigen of a virus belonging to the following non-limiting families of viruses: Retroviridae (e.g. human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; Picornaviridae (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (e.g. strains that cause

gastroenteritis); Togaviridae (e.g. equine encephalitis viruses, rubella viruses); Flaviridae (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g. coronaviruses, the etiological agent of severe acute respiratory syndrome (SARS)); Rhabdoviridae (e.g. vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g. ebola viruses); Paramyxoviridae (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g. influenza viruses); Bungaviridae (e.g. Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); Arena viridae (hemorrhagic fever viruses); Reoviridae (e.g. reoviruses, orbiviurses and rotaviruses); Birnaviridae; Hepadnaviridae (Hepatitis B virus); Parvovirida (parvoviruses); Papovaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g. African swine fever virus); and the etiological agents of Spongiform encephalopathies, the agent of delta hepatitis (a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (including class 1, internally transmitted and class 2, parenterally transmitted or Hepatitis C; Norwalk and related viruses, and astroviruses).

[0054] In other embodiments, a target antigen of the invention is an antigen of a simple retrovirus or complex retrovirus. The simple retroviruses include the subgroups of B-type retroviruses, C-type retroviruses and D-type retroviruses. An example of a B-type retrovirus is mouse mammary tumor virus (MMTV). The C-type retroviruses include subgroups C-type group A (including Rous sarcoma virus (RSV), avian leukemia virus (ALV), and avian myeloblastosis virus (AMV)) and C-type group B (including murine leukemia virus (MLV), feline leukemia virus (FeLV), murine sarcoma virus (MSV), gibbon ape leukemia virus (GALV), spleen necrosis virus (SNV), reticuloendotheliosis virus (RV) and simian sarcoma virus (SSV)). The D-type retroviruses include Mason-Pfizer monkey virus (MPMV) and simian retrovirus type 1 (SRV-1). The complex retroviruses include the subgroups of lentiviruses, T-cell leukemia viruses and the foamy viruses. Lentiviruses include HIV-1, but also include HIV-2, SIV, Visna virus, feline immunodeficiency virus (FIV), and equine infectious anemia virus (EIAV). The T-cell leukemia viruses include HTLV-1, HTLV-II, simian T-cell leukemia virus (STLV), and bovine leukemia virus (BLV). The foamy viruses include human foamy virus (HFV), simian foamy virus (SFV) and bovine foamy virus (BFV).

[0055] In certain embodiments, a target antigen of the invention is an antigen of a RNA virus that infects vertebrate animals include, but are not limited to, the following: members of the family Reoviridae, including the genus Orthoreovirus (multiple serotypes of

both mammalian and avian retroviruses), the genus Orbivirus (Bluetongue virus, Eugenangee virus, Kemerovo virus, African horse sickness virus, and Colorado Tick Fever virus), the genus Rotavirus (human rotavirus, Nebraska calf diarrhea virus, murine rotavirus, simian rotavirus, bovine or ovine rotavirus, avian rotavirus); the family Picornaviridae, including the genus Enterovirus (poliovirus, Coxsackie virus A and B, enteric cytopathic human orphan (ECHO) viruses, hepatitis A virus, Simian enteroviruses, murine encephalomyelitis (ME) viruses, Poliovirus muris, bovine enteroviruses, porcine enteroviruses, the genus Cardiovirus (Encephalomyocarditis virus (EMC), Mengovirus), the genus Rhinovirus (human rhinoviruses including at least 113 subtypes), the genus Apthovirus (Foot and Mouth disease virus (FMDV); the family Calciviridae, including Vesicular exanthema of swine virus, San Miguel sea lion virus, Feline picornavirus and Norwalk virus; the family Togaviridae, including the genus Alphavirus (Eastern equine encephalitis virus, Semliki forest virus, Sindbis virus, Chikungunya virus, O'Nyong-Nyong virus, Ross river virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus), the genus Flavirius (Mosquito borne yellow fever virus, Dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Kunjin virus, Central European tick borne virus, Far Eastern tick borne virus, Kyasanur forest virus, Louping III virus, Powassan virus, Omsk hemorrhagic fever virus), the genus Rubivirus (Rubella virus), the genus Pestivirus (mucosal disease virus, hog cholera virus, Border disease virus); the family Bunyaviridae, including the genus Bunyvirus (Bunyamwera and related viruses, California encephalitis group viruses), the genus Phlebovirus (Sandfly fever Sicilian virus, Rift Valley fever virus), the genus Nairovirus (Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus), and the genus Uukuvirus (Uukuniemi and related viruses); the family Orthomyxoviridae, including the genus Influenza virus (Influenza virus type A, many human subtypes); Swine influenza virus, and Avian and Equine Influenza viruses; influenza type B (many human subtypes), and influenza type C (possible separate genus); the family paramyxoviridae, including the genus Paramyxovirus (Parainfluenza virus type 1, Sendai virus, Hemadsorption virus, Parainfluenza viruses types 2 to 5, Newcastle Disease Virus, Mumps virus), the genus Morbillivirus (Measles virus, subacute sclerosing panencephalitis virus, distemper virus, Rinderpest virus), the genus Pneumovirus (respiratory syncytial virus (RSV), bovine respiratory syncytial virus and Pneumonia virus of mice); forest virus, Sindbis virus, Chikungunya virus, O'Nyong-Nyong virus, Ross river virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus), the genus Flavirius (Mosquito borne yellow fever virus, Dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus,

Murray Valley encephalitis virus, West Nile virus, Kunjin virus, Central European tick borne virus, Far Eastern tick borne virus, Kyasanur forest virus, Louping III virus, Powassan virus, Omsk hemorrhagic fever virus), the genus Rubivirus (Rubella virus), the genus Pestivirus (Mucosal disease virus, Hog cholera virus, Border disease virus); the family Bunyaviridae, including the genus Bunyvirus (Bunyamwera and related viruses, California encephalitis group viruses), the genus Phlebovirus (Sandfly fever Sicilian virus, Rift Valley fever virus), the genus Nairovirus (Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus); and the genus Uukuvirus (Uukuniemi and related viruses); the family Orthomyxoviridae, including the genus Influenza virus (Influenza virus type A, many human subtypes); Swine influenza virus, and Avian and Equine Influenza viruses; influenza type B (many human subtypes), and influenza type C (possible separate genus); the family paramyxoviridae, including the genus Paramyxovirus (Parainfluenza virus type 1, Sendai virus, Hemadsorption virus, Parainfluenza viruses types 2 to 5, Newcastle Disease Virus, Mumps virus), the genus Morbillivirus (Measles virus, subacute sclerosing panencephalitis virus, distemper virus, Rinderpest virus), the genus Pneumovirus (respiratory syncytial virus (RSV), Bovine respiratory syncytial virus and Pneumonia virus of mice); the family Rhabdoviridae, including the genus Vesiculovirus (VSV), Chandipura virus, Flanders-Hart Park virus), the genus Lyssavirus (Rabies virus), fish Rhabdoviruses, Marburg virus and Ebola virus; the family Arenaviridae, including Lymphocytic choriomeningitis virus (LCM), Tacaribe virus complex, and Lassa virus; the family Coronoaviridae, including Infectious Bronchitis Virus (IBV), Mouse Hepatitis virus, Human enteric corona virus, and Feline infectious peritonitis (Feline coronavirus).

[0056] In other embodiments, a target antigen of the invention is an antigen of a DNA virus that infects vertebrate animals. Encompassed are antigens of the DNA viruses belonging to the family Poxviridae, including the genus Orthopoxvirus (Variola major, Variola minor, Monkey pox Vaccinia, Cowpox, Buffalopox, Rabbitpox, Ectromelia), the genus Leporipoxvirus (Myxoma, Fibroma), the genus Avipoxvirus (Fowlpox, other avian poxvirus), the genus Capripoxvirus (sheeppox, goatpox), the genus Suipoxvirus (Swinepox), the genus Parapoxvirus (contagious postular dermatitis virus, pseudocowpox, bovine papular stomatitis virus); the family Iridoviridae (African swine fever virus, Frog viruses 2 and 3, Lymphocystis virus of fish); the family Herpesviridae, including the alpha-Herpesviruses (Herpes Simplex Types 1 and 2, Varicella-Zoster, Equine abortion virus, Equine herpes virus 2 and 3, pseudorabies virus, infectious bovine keratoconjunctivitis virus, infectious bovine rhinotracheitis virus, feline rhinotracheitis virus, infectious laryngotracheitis virus) the Beta-herpesviruses (Human cytomegalovirus and

cytomegaloviruses of swine, monkeys and rodents); the gamma-herpesviruses (Epstein-Barr virus (EBV), Marek's disease virus, Herpes saimiri, Herpesvirus ateles, Herpesvirus sylvilagus, guinea pig herpes virus, Lucke tumor virus); the family Adenoviridae, including the genus Mastadenovirus (Human subgroups A,B,C,D,E and ungrouped; simian adenoviruses (at least 23 serotypes), infectious canine hepatitis, and adenoviruses of cattle, pigs, sheep, frogs and many other species, the genus Aviadenovirus (Avian adenoviruses); and non-cultivatable adenoviruses; the family Papoviridae, including the genus Papillomavirus (Human papilloma viruses, bovine papilloma viruses, Shope rabbit papilloma virus, and various pathogenic papilloma viruses of other species), the genus Polyomavirus (polyomavirus, Simian vacuolating agent (SV-40), Rabbit vacuolating agent (RKV), K virus, BK virus, JC virus, and other primate polyoma viruses such as Lymphotrophic papilloma virus); the family Parvoviridae including the genus Adeno-associated viruses, the genus Parvovirus (feline panleukopenia virus, bovine parvovirus, canine parvovirus, Aleutian mink disease virus, etc). Finally, DNA viruses may include viruses which do not fit into the above families such as Kuru and Creutzfeldt-Jacob disease viruses and chronic infectious neuropathic agents.

[0057] In certain embodiments, a target antigen of the invention can be an antigen of bacteria which includes, but is not limited to, bacteria that have an intracellular stage in its life cycle, such as mycobacteria (e.g., Mycobacteria tuberculosis, M. bovis, M. avium, M. leprae, or M. africanum), rickettsia, mycoplasma, chlamydia, and legionella. Other examples of target antigens contemplated include but are not limited to antigens of Gram positive bacillus (e.g., Listeria, Bacillus such as Bacillus anthracis, Erysipelothrix species), Gram negative bacillus (e.g., Bartonella, Brucella, Campylobacter, Enterobacter, Escherichia, Francisella, Hemophilus, Klebsiella, Morganella, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Shigella, Vibrio, and Yersinia species), spirochete bacteria (e.g., Borrelia species including Borrelia burgdorferi that causes Lyme disease), anaerobic bacteria (e.g., Actinomyces and Clostridium species), Gram positive and negative coccal bacteria, Enterococcus species, Streptococcus species, Pneumococcus species, Staphylococcus species, Neisseria species. Specific examples of infectious bacteria include but are not limited to: Helicobacter pyloris, Borelia burgdorferi, Legionella pneumophilia, Mycobacteria tuberculosis, M. avium, M. intracellulare, M. kansaii, M. gordonae, Staphylococcus aureus, Neisseria gonorrhoeae, Neisseria meningitidis, Listeria monocytogenes, Streptococcus pyogenes (Group A Streptococcus), Streptococcus agalactiae (Group B Streptococcus), Streptococcus viridans, Streptococcus faecalis, Streptococcus bovis, Streptococcus pneumoniae, Haemophilus influenzae, Bacillus antracis,

Corynebacterium diphtheriae, Erysipelothrix rhusiopathiae, Clostridium perfringers, Clostridium tetani, Enterobacter aerogenes, Klebsiella pneumoniae, Pasturella multocida, Fusobacterium nucleatum, Streptobacillus moniliformis, Treponema pallidium, Treponema pertenue, Leptospira, Rickettsia, and Actinomyces israelli.

[0058] In other embodiments, a target antigen of the invention can be an antigen of a parasite that causes a disease in vertebrates, including human. Unicellular and multicellular parasites are contemplated. Parasites that cause these diseases can be classified based on whether they are intracellular or extracellular. An "intracellular parasite" as used herein is a parasite whose entire life cycle is intracellular. Examples of human intracellular parasites include Leishmania spp., Plasmodium spp., Trypanosoma cruzi, Toxoplasma gondii, Babesia spp., and Trichinella spiralis. An "extracellular parasite" as used herein is a parasite whose entire life cycle is extracellular. Extracellular parasites capable of infecting humans include Entamoeba histolytica, Giardia lamblia, Enterocytozoon bieneusi, Naegleria and Acanthamoeba as well as most helminths. Yet another class of parasites is defined as being mainly extracellular but with an obligate intracellular existence at a critical stage in their life cycles. Such parasites are referred to herein as "obligate intracellular parasites". These parasites may exist most of their lives or only a small portion of their lives in an extracellular environment, but they all have at least one obligate intracellular stage in their life cycles. This latter category of parasites includes Trypanosoma rhodesiense and Trypanosoma gambiense, Isospora spp., Cryptosporidium spp, Eimeria spp., Neospora spp., Sarcocystis spp., and Schistosoma spp. Thus, the invention encompasses using antigens of a parasite that causes a parasitic disease, such as but not limited to, amebiasis, malaria, leishmania, coccidia, giardiasis, cryptosporidiosis, toxoplasmosis, and trypanosomiasis, ascariasis, ancylostomiasis, trichuriasis, strongyloidiasis, toxoccariasis, trichinosis, onchocerciasis, filaria, and dirofilariasis. Also encompassed are antigens of various flukes, such as but not limited to schistosomiasis, paragonimiasis, and clonorchiasis.

[0059] Target antigens of the invention also encompass antigens of infectious agents that cause diseases in animals, especially animals of commercial interest, including but are not limited to, parasites infecting swine, e.g., Eimeria bebliecki, Eimeria scabra, Isospora suis, Giardia spp.; Balantidium coli, Entamoeba histolytica; Toxoplasma gondii and Sarcocystis spp., and Trichinella spiralis; parasites of dairy and beef cattle e.g., Eimeria spp., Cryptosporidium spp., Giardia spp., Toxoplasma gondii; Babesia bovis (RBC), Babesia bigemina (RBC), Trypanosoma spp. (plasma), Theileria spp. (RBC); Theileria parva (lymphocytes); Tritrichomonas foetus; and Sarcocystis spp; parasites of raptors e.g., Trichomonas gallinae; Eimeria spp.; Plasmodium relictum, Leucocytozoon danilewskyi

(owls), Haemoproteus spp., Trypanosoma spp.; Histomonas; Cryptosporidium meleagridis, Cryptosporidium baileyi, Giardia, Eimeria; Toxoplasma; parasites infecting sheep and goats, e.g., Eimeria spp., Cryptosporidium spp., Giardia spp.; Toxoplasma gondii; Babesia spp. (RBC), Trypanosoma spp. (plasma), Theileria spp. (RBC); and Sarcocystis spp; parasites infecting poultry e.g., Eimeria acervulina, E. necatrix, E. tenella, Isospora spp. and Eimeria truncata; Histomonas meleagridis and Histomonas gallinarum; Trichomonas gallinae; Hexamita meleagridis, Emeria maxima, Emeria meleagridis, Eimeria adenoeides, Eimeria meleagrimitis, Cryptosporidium, Eimeria brunetti, Emeria adenoeides, Leucocytozoon spp., Plasmodium spp., Hemoproteus meleagridis, Toxoplasma gondii and Sarcocystis; parasites in rodents, e.g., Leishmania spp., Plasmodium berghei, Plasmodium yoelii, Giardia muris, Hexamita muris; Toxoplasma gondii; Trypanosoma duttoni (plasma); Kiossiella muris; Sarcocystis spp, Giardia muris, Hexamita muris; Toxoplasma gondii; Trypanosoma lewisi (plasma); Trichinella spiralis; Sarcocystis spp.; parasites infecting rabbits, e.g., Eimeria spp.; Toxoplasma gondii; Nosema cuniculi; Eimeria stiedae, Sarcocystis spp., Trichomonas spp. Trichinella spiralis; Sarcocystis spp., Balantidium caviae; Klossiella caviae; Sarcocystis spp.; parasites of birds, e.g., Trichomonas gallinae; Eimeria spp., Isospora spp., Giardia; Cryptosporidium; Sarcocystis spp., Toxoplasma gondii, Haemoproteus/Parahaemoproteus, Plasmodium spp., LeucocytozoonlAkiba, Atoxoplasma, Trypanosoma spp.; parasites infecting dogs, e.g., Trichinella spiralis; Isopora spp., Sarcocystis spp., Cryptosporidium spp., Hammondia spp., Giardia duodenalis (canis); Balantidium coli, Entamoeba histolytica; Hepatozoon canis; Toxoplasma gondii, Trypanosoma cruzi; Babesia canis, Leishmaniá amastigotes; Neospora caninum; parasites infecting feline species, e.g., Isospora spp., Toxoplasma gondii, Sarcocystis spp., Hammondia hammondi, Besnoitia spp., Giardia spp.; Entamoeba histolytica; Hepatozoon canis, Cytauxzoon spp., Cytauxzoon spp., Cytauxzoon spp.; parasites infecting fish include Hexamita spp., Eimeria spp.; Cryptobia spp., Nosema spp., Myxosoma spp., Chilodonella spp., Trichodina spp.; Plistophora spp., Myxosoma Henneguya; Costia spp., Ichthyophithirius spp., and Oodinium spp.; parasites infecting horses, e.g., Gasterophilus spp.; Eimeria leuckarti, Giardia spp.; Tritrichomonas equi; Babesia spp. (RBC's), Theileria equi; Trypanosoma spp.; Klossiella equi; Sarcocystis spp.; parasites of wild mammals include Giardia spp. (carnivores, herbivores), Isospora spp. (carnivores), Eimeria spp. (carnivores, herbivores); Theileria spp. (herbivores), Babesia spp. (carnivores, herbivores), Trypanosoma spp. (carnivores, herbivores); Schistosoma spp. (herbivores); Fasciola hepatica (herbivores), Fascioloides magna (herbivores), Fasciola gigantica (herbivores), Trichinella spiralis (carnivores, herbivores); parasites of the Bovidae family (blesbok,

antelope, banteng, eland, gaur, impala, klipspringer, kudu, gazelle) include Eimeria spp; typical parasites in the Pinnipedae family (seal, sea lion) include Eimeria phocae; typical parasites in the Camelidae family (camels, llamas) include Eimeria spp; typical parasites of the Giraffidae family (giraffes) include Eimeria spp.; typical parasites in the Elephantidae family (African and Asian) include Fasciola spp.; typical parasites of lower primates (chimpanzees, orangutans, apes, baboons, macaques, monkeys) include Giardia spp.; Balantidium coli, Entamzoeba histolytica, Sarcocystis spp., Toxoplasma gondii; Plasmodim spp.

[0060] Preferred examples of target antigens useful for treatment of viral infections include but are not limited to the envelope proteins, gag proteins, gp120, gp160, p24, nef, vpr, tat proteins, and reverse transcriptase of retroviruses, including various strains of human immunodeficiency virus (HIV); the L1, L2, E1, E6 and E7 proteins of various subtypes of human papillomavirus (HPV); VP16, VP26, RS1, UL11, UL13, UL18, UL33, UL36, UL37, UL40, UL41, UL45, UL46, UL49, UL54, US9, US11, RL2, , RS1, UL1, UL10, UL14, UL16, UL17, UL20, UL22, UL27, UL34, UL36, UL37, UL44, UL48, UL53, US5, US6, US9, US10, US11 and the glycoproteins - gB, gC, gD, gH, gJ, gK, gL, gM of herpes simplex virus types I and II; spike prtoetin, nucleocapsid protein of severe acute respiratory syndrome coronavirus (SARS-CoV); and haemagglutinins (H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16) and neuramidases (N1, N2, N3, N4, N5, N6, N7, N8, N9) of various subtypes of influenza virus A, B, or C that infect humans and animals including birds.

[0061] Other non-limiting examples of target antigens include O, H and K proteins of Vibrio cholerae, Shiga toxin-producing E. coli, and Enterotoxigenic E. coli; S-Hp and Hp-lC for Heliobacter pylori catalase; HpaA, Ompl8, groEL, ureB, ureA, Hypo. ORF, and napA for H. pylori; RRV-Gl, RRV-G2, RRV-G4, VP4, VP7 and G antigen for Rotavirus spp.; capsid proteins, SV, NV and MXV for Calavirus; O, H, K, and fimbrial antigen for Shigella spp.; O, V, H, K, and X antigens for S. typhii; O, H, and K antigens for Campylobacter spp.; A antigen and BAD1 for Blastomycosis; mannoprotein antigen for Candidiasis; Ag2 and TRCP for Coccidiodiomycosis; capsule proteins for Cryptococcosis; HIS-G2 and H antigen for Histoplasmosis; gp43 and PIO for Paracoccidiodiomycosis; gB, gH, UL83 for Cytomegalovirus; gB 350/220 for Epstein-Barr virus; TRAP/SSP2, LSA -1, LSA -3, MSP1, AMA1, EBA 175, DBA, MSP3, GLURP, MSP1, MSP2, RESA, and NYVAC - PF7 for Plasmodium spp.; GST, TP1 and MAPS for Schistosomiasis; M protein, SCPA, SPE, SPE B and SfbI for Group A Streptococci; CPS, C Protein, Rib Protein for Group B Streptococci; Sip, V protein and HibPS for Haemophilus influenzae Type B; OMP

P2, PG OMP, LOS, HMW1, HMW2, and hia for nontypeable Hemophilus influenzae; H, F, M, and N proteins for Paramyxovirus; BI, LBP, USPA1, OMPCD, OMPE and OMP B2 for Moraxella catarrhalis; H, N and F for Parainfluenza virus; F, G, and M proteins, F and G glycoproteins, and BBG2Na for respiratory synctial virus; PspA, PspC, autolysin, pneumolysin, hyaluronate lyase, surface antigen A, choline binding protein A and neuraminidase enzymes for Streptococcus pneumoniae; toxin fragment, H, O, HA, and CT for C. tetanii; Ag 85B for M. tuberculosis; porin protein for N. gonorrhoeae; gB and gD for Herpes simplex virus; E6 and E7 for human papilloma virus; protective antigen (PA, including PA83, PA63, PA20), spore antigens, lethal factor (LF), edema factor (EF), and combinations of PA, LF and/or EF for Bacillus anthracis; HBsAg, HBcAg, HBeAg, ORF 2 for hepatitis E, and anti-HBC for hepatitis B Virus; envelope glycoprotein and viral core protein for hepatitis C virus.

[0062] Other non-limiting examples of target antigens include Mip and LIGA for Legionella pneumophilia; OSPA, flagellins, OSP B and VISE for Borelia burgdorferi; C-mycosides, A,D, lipoarabino-mannan and GI-ai for Mycobacterium avium; MCMP, GI-ai for M. intracellulare; GI-ai for M. Kansaii, protein A for Staphylococcus aureus; H and and O antigens for L. Monocytogenes; A, T1, T2, rSb28 GST, RSm28GST, rSh28GST and rSbSWAP for S. bovis; EFSI, EFS2, EFM3, EFM4, EFM5 and C130 for Entercocus spp.; K and O antigens for C. Diptheriae; Spa A, surface proteins, and peptidoglycan for E. rhusiopathiae; H and O antigens for C. perfringers; J, K, and O for E. aerogenes. O, K, and LPs for Klebsiella pneumoniae; capsule and LPS for Pseudomonas multocida; capsule and surface antigens for Bacteroides spp.; O and H antigens for S. moniliformis; TmpC, TmpA and TpD for T. pallidium; TrpK, Tp92, and Gpd for T. pertenue; cell surface antigens for Leptospira; rpLs and OMP for Rickettsia; capsule, cytoplasmic and surface antigens for Cryptococcus neoformans; H and M antigens for H. capsulatum; F, TP and CF for C. immitis; A antigen for B. dermatitidis. Enolase, CT579, P242, and TroA for Chlamydia trachomatis; HxK2P, Pg1p, Tpi7p, Gap1p, Eno1p, and Adh1p for C. albicans; CSP, MSA, SPAM, LSA TPA, S-antigen, GBP, HRP, ABRA, RESA, MESA and FIRPA for Plasmodium sp; GRA 1, GRA 7 and ROP 2 for Trypanosoma gondii; GST-12P3, GST-11c5, and BGT for Babesia spp.; SLA rgp 63, rk39, gene B proteins, rHZA, rHZB, rLACK, rPSA, and rP20 for Leishmania sp; CRA, FRA and Cd1d for Trypanosoma cruzi.

[0063] Antigens of viral pathogens that infect aquaculture can be used as target antigens, and include but are not limited to glycoprotein (G) or nucleoprotein (N) of viral hemorrhagic septicemia virus (VHSV); G or N proteins of infectious hematopoietic necrosis virus (IHNV); VP1, VP2, VP3 or N structural proteins of infectious pancreatic necrosis

virus (IPNV); G protein of spring viremia of carp (SVC); a membrane-associated protein, tegumin or capsid protein or glycoprotein of channel catfish virus (CCV); an iron-regulated outer membrane protein, (IROMP), an outer membrane protein (OMP), and an A-protein of Aeromonis salmonicida which causes furunculosis, p57 protein of Renibacterium salmoninarum which causes bacterial kidney disease (BKD), major surface associated antigen (msa), a surface expressed cytotoxin (mpr), a surface expressed hemolysin (ish), and a flagellar antigen of Yersiniosis; an extracellular protein (ECP), an iron-regulated outer membrane protein (IROMP), and a structural protein of Pasteurellosis; an OMP and a flagellar protein of Vibrosis anguillarum and V. ordalii; a flagellar protein, an OMP protein, aroA, and purA of Edwardsiellosis ictaluri and E. tarda; and surface antigen of Ichthyophthirius; and a structural and regulatory protein of Cytophaga columnari; and a structural and regulatory protein of Rickettsia; surface antigens of Ichthyophthirius.

[0064] In a specific embodiment, a target antigen of the invention is not an antigen of a pathogen or infectious agent. In another specific embodiment, a target antigen of the invention is not an antigen of herpes virus simplex virus, herpes virus simplex virus I, or herpes virus simplex virus II. In specific embodiments, one or more of the following antigens of herpes simplex virus type I and II is not a target antigen: RL2, RS1, UL1, UL10, UL13, UL14, UL16, UL17, UL20, UL22, UL27, UL33, UL34, UL36, UL37, UL40, UL41, UL44, UL45, UL46, UL48, UL49, UL53, UL54, US5, US6, US9, US10, and/or US11.

[0065] In other embodiments, metabolic disorders other than cancer and infectious diseases that can be treated or prevented by the compositions and methods of the invention include, for example, cardiovascular disorders, hormonal disorders, and neurological disorders. In a specific embodiment, the compositions and methods of the invention can be used for fertility management or contraception. Generally, in these embodiments, a target antigen is a protein present in a subject that is known or suspected to play a role in the mechanism, progression, pathogenicity, pathology, and/or symptoms of a metabolic disorder, such as but not limited to obesity, hypercholesterolemia, hypertension, osteoporosis, rheumatoid arthritis, psoriasis, or atherosclerosis, Alzheimer's disease, and dementia. For many disorders, such proteins have been identified and can be or have been targeted by one or more drugs to produce a therapeutic or prophylactic benefit in the subject. For some metabolic disorders, the target antigen may comprise or consist essentially of an aberrant form of a normal cellular protein, such as a prion or an amyloid beta protein. In the present invention, such a protein is used as a target antigen such that an immune response can be elicited in a subject against the protein, resulting in suppression of one or more functions of the protein, reduction of the level of the protein, and/or reduction in the number

or cells producing or bearing the protein. Nonlimiting examples of a target antigen include but is not limited to, ghrelin, angiotensin II, RANKL, cholesterol ester transfer protein, TNF-alpha, and follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), lutenizing hormone (LH), and their subunits.

[0066] The term "antigenic cell" refers to any cell of a multicellular organism or a single cell organism that comprises a target antigen. In various embodiments, the purified target antigen preparation can be obtained from antigenic cells, a cellular fraction of antigenic cells, or virus particles. The purified target antigen preparation can be obtained from a cellular fraction, such as the cytosol. The target antigen can also be a non-cytosolic protein (e.g., one from cell walls, cell membranes or organelles) present in cellular fractions such as but are not limited to cytosolic fractions, membrane fractions, and organelle fractions, such as nuclear, mitochondrial, lysosomal, and endoplasmic reticulum-derived fractions. The target antigen preparation can be made from non-recombinant or recombinant cells. The target antigen preparation obtained from the antigenic cells or cellular fractions thereof or virus particles can be purified by any technique known in the art.

[0067] A target antigen of the invention can be recombinant or non-recombinant and can be obtained by many methods known in the art, such as but not limited to chemical synthesis, in vitro translation of a target antigen nucleic acid, recombinant expression of a cloned target antigen nucleic acid in a host cell, or purification from cancer cells, infected cells, or pathogens.

[0068] A preparation of target antigen can be made using well known peptide synthesis procedures, as described in e.g., Merrifield, Science 232:341-347 (1986); Barany and Merrifield, The Peptides, Gross and Meienhofer, eds. (New York, Academic Press), pp. 1-284 (1979); and Stewart and Young, Solid Phase Peptide Synthesis, (Rockford, Ill., Pierce), 2d Ed. (1984), which are incorporated by reference herein.

[0069] In another embodiment, a preparation of target antigen can be made with target antigen nucleic acids using well known methods of in vitro translation. In yet another embodiment, a preparation of target antigen can be made with target antigen nucleic acids using well known methods of recombinant expression in a host cell. Techniques and reagents used for recombinant expression of HSP and α2M described in Section 5.3.7.1 can also be used to obtain a purified target antigen preparation. As used herein, the term "target antigen nucleic acid" refers to any nucleic acid comprising a nucleotide sequence which encodes a target antigen.

[0070] In yet another embodiment, the target antigen from antigenic cells can be purified by its general biochemical and/or biophysical properties, such as size, density, charge, cellular location or combinations thereof. To make a target antigen preparation of antigenic cells or virus particles, the lysing of antigenic cells or disruption of cell walls, cell membranes, or viral particle structure can be performed using standard protocols known in the art. These methods can also be applied to recombinant host cells which express the target antigen.

[0071] As used herein, the term "purified target antigen preparation" refers to a composition consisting essentially of a single protein or polypeptide to which an immune response in a subject is desired, or comprising a purified protein or polypeptide to which an immune response in a subject is desired. The term "purified" when applied to a target antigen preparation denotes that the target antigen is the predominant species of protein or polypeptide in the preparation. Preferably, the preparation is also essentially free of other non-proteinaceous materials, such as those that are associated with the target antigen in the natural state.

[0072] The target antigen of the present invention can be purified to substantial homogeneity by standard techniques well known in the art, including, for example, selective precipitation with salts such as ammonium sulfate; ion exchange chromatography; size exclusion chromatography; isoelectric focusing; high performance liquid chromatography (HPLC); immunopurification methods, and other purification techniques. See, e.g., Scopes, Protein Purification: Principles and Practice (Springer-Verlag: New York (1982)), which are incorporated herein by reference. The purity of the target antigen preparation may be determined by any means known in the art.

[0073] A purified target antigen preparation may comprise greater than about 80% by weight protein of the target antigen of interest, more preferably greater than about 90% by weight protein of the target antigen of interest, more preferably greater than about 95% by weight protein of the target antigen of interest, more preferably greater than about 97% by weight protein of the antigen of interest and/or less than about 3% by weight of other proteins, even more preferably greater than about 99% by weight protein of the antigen of interest and/or less than about 1% by weight of other proteins, and most preferably greater than about 99.5% by weight protein of the antigen of interest and/or less than about 0.5% by weight of other proteins.

[0074] In one embodiment, the purity of the target antigen preparation is determined by examining a sample of the preparation by polyacrylamide gel electrophoresis (PAGE).

The term "purified" in this context denotes that the preparation gives rise to a single band at a position corresponding to that of the target antigen in the gel after electrophoresis.

[0075] In another embodiment, the purity of the target antigen preparation is determined by mass spectrometry (MS). The term "purified" in this context denotes that the preparation gives rise to a single predominant peak in the mass spectrum at a position corresponding to that of an ionized form of the target antigen. Preferably, the relative ion intensity of the peak corresponding to the target antigen is greater than 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 95%, 99%. More preferably, the relative ion intensity of the predominant peak corresponding to the target antigen is at least 30%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, 95% greater than that of a peak with the next highest relative ion intensity and that is unrelated to the target antigen.

[0076] A purified target antigen preparation can be used to prepare the peptides for complexing to HSP or α2M.

[0077] In a preferred embodiment, different purified target antigen preparations, for example up to 2, 3, 4, 5, 10, or 15 different purified target antigen preparations, can be combined prior to cleavage and complexing, wherein the target antigen is different in each of the two or more purified target antigen preparations being combined.

5.2. ANTIGENIC PEPTIDES

[0078] According to the invention, a purified target antigen preparation is subjected to one or more methods of the invention to generate an antigenic set of peptides. The set of peptides comprises a plurality of peptides corresponding to various overlapping and/or non-overlapping segments of the target antigen, and can be of different lengths, amino acid sequences, and amino acid compositions. A peptide in the set may comprise one or more different epitopes that are present in the target antigen. The individual peptides in the set may display different immunogenicities and antigenicities. Preferably, all the different peptides in a set are antigenic. Therefore, while the set of peptides are antigenic and produces antigenic complexes when complexed to HSP or α2M, not every peptide in the set is necessarily antigenic or immunogenic. The term "an antigenic set of peptides" is used herein collectively to refer to the set of peptides (1) that results from a process which fragments the target antigen in a purified target antigen preparation, and (2) that is antigenic or immunogenic or produces antigenic or immunogenic complexes when complexed to HSP or α2M. An antigenic set of peptides may be purified after the fragmentation process. An antigenic set of peptides comprises a plurality of different peptides, for example, at least 2, 5, 10, 15, 20, 25, 30, 40, 50, 100, 250, or 500 different peptides, depending on the size of the target antigen, the frequency of cleavage, and the extent of the reaction.

[0079] Many methods known in the art can be used to cleave or fragment the target antigen to generate an antigenic set of peptides. A proteolytic composition comprising one or more proteolytic agents, such as enzymes and/or chemical cleavage agents can be used to cleave or fragment the target antigen to generate an antigenic set of peptides.

[0080] In one embodiment, an enzymatic reaction is used to generate an antigenic set of peptides from a target antigen. Preferably, a protease is used. The term protease as used herein is synonymous with proteinase, peptidase, or proteolytic enzyme. In certain embodiments, a protein complex that shows protease activity, or a proteosome can be used. Most preferably, an endoprotease (or endopeptidase) is used. The enzymatic digestion can be performed either individually or in suitable combinations with any of the proteases that are well known in the art including, but not limited to, trypsin, Staphylococcal peptidase I (also known as protease V8), chymotrypsin, pepsin, cathepsin G, thermolysin, elastase, peptidylglutamylpeptide-hydrolase, the caspases, and papain. For example, trypsin is a highly specific serine proteinase that cleaves on the carboxyl-terminal side of lysines and arginines. Due to the limited number of cleavage sites, it is expected to leave many MHC-binding epitopes intact. Staphylococcal peptidase I, a serine proteinase, has specificity for cleavage after glutamic and aspartic acid residues. The peptide sets resulting from these enzymatic digestion reactions comprise antigenic peptides, and possibly non-antigenic peptides, and single amino acid residues. The amino acid sequences of the peptides in the set are subsequences of the amino acid sequence of the target antigen. Information and software tools regarding proteases, their cleavage site specificities, and reaction conditions can be found in the MEROPS database Release 6.7 (Rawlings, N.D., O'Brien, E. A. & Barrett, A.J. (2002) MEROPS: the protease database. Nucleic Acids Res. 30, 343-346); Keil, B. Specificity of proteolysis. Springer-Verlag Berlin-Heidelberg-NewYork, pp.335. (1992); Barrett A., Rawlings N.D., Woessner J.F. Handbook of proteolytic enzymes. Academic Press (1998); PeptideCutter software, see Gasteiger E., Gattiker A., Hoogland C., Ivanyi I., Appel R.D., Bairoch A.ExPASy: the proteomics server for in-depth protein knowledge and analysis, Nucleic Acids Res. 31:3784-3788(2003); which are incorporated herein by reference in their entireties.

[0081] In another embodiment, non-enzymatic methods are used. Such methods comprise cleavage by a chemical compound, such as but not limited to cyanogen bromide (CNBr), hydroxylamine, or iodosobenzoic acid. See Han, K.K et al., Current develpoments in chemical cleavage of proteins. International Journal of Biochemistrey 15: 875-884, which is incorporated herein by reference in its entirety.

[0082] In various embodiments, it may not be necessary to completely digest or cleave the proteins in the reactions. Depending on the proteins, enzymes and reaction conditions, undigested target antigen may remain in the reactions. These reactions may comprise undigested and incompletely digested polypeptides of the target antigen. The reaction conditions may be adjusted to produce peptides of a desired size distribution. These reactions may result in the generation of a greater diversity of peptides of the target antigen than reactions that are complete. In certain embodiments, the reaction conditions can also be manipulated to produce different digestion patterns using a cleavage agent.

[0083] Different enzymes and chemicals will generate distinct sets of peptides. In another embodiment, the target antigen preparation to be fragmented can be aliquoted into a plurality of reactions each using a different enzyme or chemical, and the resulting sets of peptides may optionally be pooled together for complexing to HSP or α2M. In yet another embodiment, the target antigen may be treated with two or more fragmentation or cleavage agents sequentially, or simultaneously if the reaction conditions permit. Different enzymes can be used. Different chemical agents can be used. A combination of enzymes and chemical agents can also be used. The production of different peptide sets by pooling separate digestions using different cleavage agents and conditions, or by double/multiple digestion(s) allow for a greater probability of generating antigenic peptides that are capable of inducing a strong immune response to the target antigen when they are complexed to HSP or α2M.

[0084] In a preferred embodiment, the target antigen preparation to be digested is aliquoted into two separate reactions and two different proteolytic enzymes/chemicals are used to produce two different sets of peptides of the proteins present in the target antigen. The fragmentation reactions of the invention are monitored in order to generate peptides that fall within a desirable range of lengths. In a preferred embodiment, the peptides generated are from about 7 to about 20 amino acid residues. Most antigenic peptides that are presented to T cells by MHC class I and class II fall within this range. In various embodiments, the antigenic set of peptides comprises peptides having a size range of 6 to 21, 8 to 19, 10 to 20, or at least 7, 8, 9, 10, 11, 12, 15, 20, 25, 30, 40, 45, or 50, amino acid residues. In preferred embodiments, the antigenic peptides within the antigenic set of peptides have 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid residues.

[0085] Once the proper conditions are established for the generation of peptide fragments of a particular size range from a target antigen, the reaction conditions can be duplicated to generate sets of antigenic peptides which can be pooled. It is preferred that the reaction is terminated before the peptides are complexed to HSPs or α2M. Any method

known in the art for terminating the reaction can be used, including but not limited to use of protease inhibitors. At the end of the digestion reaction, the peptides can optionally be separated from low molecular weight materials, such as dipeptides, or single amino acid residues, in the preparation. For example, the peptides can be isolated by centrifugation through a membrane, such as the Centriprep-3. Optionally, the peptides can be purified by their general biochemical and/or biophysical properties, such as size, charge, or combinations thereof.

5.3. PREPARATION OF HSPs AND α2M

[0086] According to the present invention, antigenic set of peptides derived from target antigens are complexed to HSPs and/or α2M. HSPs and α2M from any vertebrate species, mammalian species, and preferably human, can be used. Described herein are exemplary methods that can be used for isolating and preparing HSPs and α2M for use in the invention.

[0087] Heat shock proteins, which are also referred to interchangeably herein as stress proteins, useful in the practice of the instant invention can be selected from among any cellular protein that satisfies the following criteria. It is capable of binding other proteins or peptides, it is capable of releasing the bound proteins or peptides in the presence of adenosine triphosphate (ATP) or under acidic conditions; and it either is a protein whose intracellular concentration increases when a cell is exposed to a stressful stimuli, or it is a protein (e.g., a constitutive homolog of the foregoing) showing at least 35% homology with any cellular protein having the above properties.

[0088] The first stress proteins to be identified were the heat shock proteins (HSPs). As their name implies, HSPs are synthesized by a cell in response to heat shock. To date, five major classes of HSPs have been identified, based on the molecular weight of the family members. These classes are called sHSPs (small heat shock proteins), HSP60, HSP70, HSP90, and HSP100, where the numbers reflect the approximate molecular weight of the HSPs in kilodaltons. In addition to the major HSP families, an endoplasmic reticulum resident protein, calreticulin, has also been identified as yet another heat shock protein useful for eliciting an immune response when complexed to antigenic molecules (Basu and Srivastava, 1999, J. Exp. Med. 189:797-202). Other stress proteins that can be used in the invention include but are not limited to grp78 (or BiP), protein disulphide isomerase (PDI), HSP110, and grp170 (Lin et al., 1993, Mol. Biol. Cell, 4:1109-1119; Wang et al., 2001, J. Immunol., 165:490-497). Many members of these families were found subsequently to be induced in response to other stressful stimuli including, but not limited to, nutrient deprivation, metabolic disruption, oxygen radicals, hypoxia and infection with

intracellular pathogens. (See Welch, May 1993, Scientific American 56-64; Young, 1990, Annu. Rev. Immunol. 8:401-420; Craig, 1993, Science 260:1902-1903; Gething, et al., 1992, Nature 355:33-45; and Lindquist, et al., 1988, Annu. Rev. Genetics 22:631-677), the disclosures of which are incorporated herein by reference. It is contemplated that HSPs/stress proteins belonging to all of these families can be used in the practice of the instant invention.

[0089] The major HSPs can accumulate to very high levels in stressed cells, but they occur at low to moderate levels in cells that have not been stressed. For example, the highly inducible mammalian HSP70 is hardly detectable at normal temperatures but becomes one of the most actively synthesized proteins in the cell upon heat shock (Welch, et al., 1985, J. Cell. Biol. 101:1198-1211). In contrast, HSP90 and HSP60 proteins are abundant at normal temperatures in most, but not all, mammalian cells and are further induced by heat (Lai, et al., 1984, Mol. Cell. Biol. 4:2802-10; van Bergen en Henegouwen, et al., 1987, Genes Dev. 1:525-31).

[0090] Heat shock proteins are among the most highly conserved proteins in existence. For example, DnaK, the HSP70 from E. coli has about 50% amino acid sequence identity with HSP70 proteins from excoriates (Bardwell, et al., 1984, Proc. Natl. Acad. Sci. 81:848-852). The HSP60 and HSP90 families also show similarly high levels of intrafamilies conservation (Hickey, et al., 1989, Mol. Cell. Biol. 9:2615-2626; Jindal, 1989, Mol. Cell. Biol. 9:2279-2283). In addition, it has been discovered that the HSP60, HSP70 and HSP90 families are composed of proteins that are related to the stress proteins in sequence, for example, having greater than 35% amino acid identity, but whose expression levels are not altered by stress. Therefore it is contemplated that the definition of heat shock protein or stress protein, as used herein, embraces other proteins, muteins, analogs, and variants thereof having at least 35% to 55%, preferably 55% to 75%, and most preferably 75% to 85% amino acid identity with members of the three families whose expression levels in a cell are enhanced in response to a stressful stimulus.

[0091] In an embodiment wherein the HSP portion of the HSP-antigenic peptide complex is desired to be purified from cells, exemplary purification procedures such as described in Sections 5.3.1- 5.3.3 below can be employed to purify HSP-peptide complexes, after which the HSPs can be separated from the endogenous HSP-peptide complexes in the presence of ATP or under acidic conditions, for subsequent in vitro complexing to an antigenic set of peptides. See Peng, et al., 1997, J. Immunol. Methods, 204:13-21; Li and Srivastava, 1993, EMBO J. 12:3143-3151, which are incorporated herein by reference in their activities. Although described for tumor cells, the protocols described hereinbelow

may be used to isolate HSPs from any infected cells, and any eukaryotic cells, for example, tissues, isolated cells, or immortalized eukaryote cell lines infected with an intracellular pathogen, tumor cells or tumor cell lines.

5.3.1. PREPARATION AND PURIFICATION OF HSP70

[0092] The purification of HSP70-peptide complexes has been described previously, see, for example, Udono et al., 1993, J. Exp. Med. 178:1391-1396. A procedure that may be used, presented by way of example but not limitation, is described below.

[0093] Initially, tumor cells are suspended in 3 volumes of 1X Lysis buffer consisting of 30mM sodium bicarbonate pH 7.5, 1mM PMSF. Then, the pellet is sonicated, on ice, until >99% cells are lysed as determined by microscopic examination. As an alternative to sonication, the cells may be lysed by mechanical shearing by homogenizing the cells in a Dounce homogenizer until >95% cells are lysed.

[0094] Then the lysate is centrifuged at 1,000g for 10 minutes to remove unbroken cells, nuclei and other cellular debris. The resulting supernatant is recentrifuged at 100,000g for 90 minutes, the supernatant harvested and then mixed with Con A Sepharose equilibrated with phosphate buffered saline (PBS) containing 2mM Ca^{2+} and 2mM Mg^{2+}. When the cells are lysed by mechanical shearing the supernatant is diluted with an equal volume of 2X lysis buffer prior to mixing with Con A Sepharose. The supernatant is then allowed to bind to the Con A Sepharose for 2-3 hours at 4°C. The material that fails to bind is harvested and dialyzed for 36 hours (three times, 100 volumes each time) against 10mM Tris-Acetate pH 7.5, 0.1 mM EDTA, 10mM NaCl, 1mM PMSF. Then the dialyzate is centrifuged at 17,000 rpm (Sorvall SS34 rotor) for 20 minutes. Then the resulting supernatant is harvested and applied to a Mono Q FPLC column equilibrated in 20mM Tris-Acetate pH 7.5, 20mM NaCl, 0.1mM EDTA and 15mM 2-mercaptoethanol. The column is then developed with a 20mM to 500mM NaCl gradient and then eluted fractions fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and characterized by immunoblotting using an appropriate anti-HSP70 antibody (such as from clone N27F3-4, from StressGen).

[0095] Fractions strongly immunoreactive with the anti-HSP70 antibody are pooled and the HSP70 protein is precipitated with ammonium sulfate; specifically with a 50%-70% ammonium sulfate cut. The resulting precipitate is then harvested by centrifugation at 17,000 rpm (SS34 Sorvall rotor) and washed with 70% ammonium sulfate. The washed precipitate is then solubilized and any residual ammonium sulfate removed by gel filtration on a Sephadex^R G25 column (Pharmacia). If necessary the HSP70 preparation thus obtained can be repurified through the Mono Q FPLC Column as described above.

[0096] The HSP70 can be purified to apparent homogeneity using this method. Typically 1 mg of HSP70 can be purified from 1 g of cells/tissue.

[0097] An improved method for purification of HSP70 comprises contacting cellular proteins with ATP or a nonhydrolyzable analog of ATP affixed to a solid substrate, such that HSP70 in the lysate can bind to the ATP or nonhydrolyzable ATP analog, and eluting the bound HSP70. A preferred method uses column chromatography with ATP affixed to a solid substratum (e.g., ATP-agarose). The resulting HSP70 preparations are higher in purity and devoid of contaminating peptides. The HSP70 yields are also increased significantly by about more than 10 fold.

[0098] Alternatively, chromatography with nonhydrolyzable analogs of ADP, instead of ATP, can be used for purification of HSP70-peptide complexes. By way of example but not limitation, purification of HSP70 free of peptide by ATP-agarose chromatography can be carried out as follows:

[0099] Meth A sarcoma cells (500 million cells) are homogenized in hypotonic buffer and the lysate is centrifuged at 100,000 g for 90 minutes at 4°C. The supernatant is applied to an ATP-agarose column. The column is washed in buffer and is eluted with 5 column volumes of 3 mM ATP. The HSP70 elutes in fractions 2 through 10 of the total 15 fractions which elute. The eluted fractions are analyzed by SDS-PAGE. The HSP70 can be purified to apparent homogeneity using this procedure.

5.3.2. PREPARATION AND PURIFICATION OF HSP90

[00100] A procedure that can be used, presented by way of example but not limitation, is described below.

[00101] Initially, tumor cells are suspended in 3 volumes of 1X Lysis buffer consisting of 30mM sodium bicarbonate pH 7.5, 1mM PMSF. Then, the pellet is sonicated, on ice, until >99% cells are lysed as determined by microscopic examination. As an alternative to sonication, the cells may be lysed by mechanical shearing by homogenizing the cells in a Dounce homogenizer until >95% cells are lysed.

[00102] Then the lysate is centrifuged at 1,000g for 10 minutes to remove unbroken cells, nuclei and other cellular debris. The resulting supernatant is recentrifuged at 100,000g for 90 minutes, the supernatant harvested and then mixed with Con A Sepharose equilibrated with PBS containing 2mM Ca^{2+} and 2mM Mg^{2+}. When the cells are lysed by mechanical shearing the supernatant is diluted with an equal volume of 2X Lysis buffer prior to mixing with Con A Sepharose. The supernatant is then allowed to bind to the Con A Sepharose for 2-3 hours at 4°C. The material that fails to bind is harvested and dialyzed for 36 hours (three times, 100 volumes each time) against 20mM sodium phosphate pH 7.4,

1 mM EDTA, 250mM NaCl. Then the dialyzate is centrifuged at 17,000 rpm (Sorvall SS34 rotor) for 20 minutes. Then the resulting supernatant is harvested and applied to a Mono Q FPLC column equilibrated with dialysis buffer. The proteins are then eluted with a salt gradient of 200mM to 600mM NaCl.

[00103] The eluted fractions are fractionated by SDS-PAGE and fractions containing HSP90 are identified by immunoblotting using an anti-HSP90 antibody such as 3G3 (Affinity Bioreagents). HSP90 can be purified to apparent homogeneity using this procedure. Typically, 150-200 μg of HSP90 can be purified from 1g of cells/tissue.

5.3.3. PREPARATION AND PURIFICATION OF GP96-PEPTIDE COMPLEXES

[00104] A procedure that can be used, presented by way of example but not limitation, is described below.

[00105] A pellet of tumors is resuspended in 3 volumes of buffer consisting of 30mM sodium bicarbonate buffer (pH 7.5) and 1mM PMSF and the cells allowed to swell on ice 20 minutes. The cell pellet is then homogenized in a Dounce homogenizer (the appropriate clearance of the homogenizer will vary according to each cell type) on ice until >95% cells are lysed.

[00106] The lysate is centrifuged at 1,000g for 10 minutes to remove unbroken cells, nuclei and other debris. The supernatant from this centrifugation step is then recentrifuged at 100,000g for 90 minutes. The gp96 protein can be purified either from the 100,000 pellet or from the supernatant.

[00107] When purified from the supernatant, the supernatant is diluted with equal volume of 2X lysis buffer and the supernatant mixed for 2-3 hours at 4°C with Con A Sepharose equilibrated with PBS containing 2mM Ca^{2+} and 2mM Mg^{2+}. Then, the slurry is packed into a column and washed with 1X lysis buffer until the OD_{280} drops to baseline. Then, the column is washed with 1/3 column bed volume of 10% α-methyl mannoside (α-MM) dissolved in PBS containing 2mM Ca^{2+} and 2mM Mg^{2+}, the column sealed with a piece of parafilm, and incubated at 37°C for 15 minutes. Then the column is cooled to room temperature and the parafilm removed from the bottom of the column. Five column volumes of the α-MM buffer are applied to the column and the eluate analyzed by SDS-PAGE. Typically the resulting material is about 60-95% pure, however this depends upon the cell type and the tissue-to-lysis buffer ratio used. Then the sample is applied to a Mono Q FPLC column (Pharmacia) equilibrated with a buffer containing 5mM sodium phosphate, pH 7. The proteins are then eluted from the column with a 0-1M NaCl gradient and the gp96 fraction elutes between 400mM and 550mM NaCl.

[00108] The procedure, however, may be modified by two additional steps, used either alone or in combination, to consistently produce apparently homogeneous gp96 preparations. One optional step involves an ammonium sulfate precipitation prior to the Con A purification step and the other optional step involves DEAE-Sepharose purification after the Con A purification step but before the Mono Q FPLC step.

[0100] In the first optional step, described by way of example as follows, the supernatant resulting from the 100,000g centrifugation step is brought to a final concentration of 50% ammonium sulfate by the addition of ammonium sulfate. The ammonium sulfate is added slowly while gently stirring the solution in a beaker placed in a tray of ice water. The solution is stirred from about ½ to 12 hours at 4°C and the resulting solution centrifuged at 6,000 rpm (Sorvall SS34 rotor). The supernatant resulting from this step is removed, brought to 70% ammonium sulfate saturation by the addition of ammonium sulfate solution, and centrifuged at 6,000 rpm (Sorvall SS34 rotor). The resulting pellet from this step is harvested and suspended in PBS containing 70% ammonium sulfate in order to rinse the pellet. This mixture is centrifuged at 6,000 rpm (Sorvall SS34 rotor) and the pellet dissolved in PBS containing 2mM Ca^{2+} and Mg^{2+}. Undissolved material is removed by a brief centrifugation at 15,000 rpm (Sorvall SS34 rotor). Then, the solution is mixed with Con A Sepharose and the procedure followed as before.

[0101] In the second optional step, described by way of example as follows, the gp96 containing fractions eluted from the Con A column are pooled and the buffer exchanged for 5mM sodium phosphate buffer, pH 7, 300mM NaCl by dialysis, or preferably by buffer exchange on a Sephadex G25 column. After buffer exchange, the solution is mixed with DEAE-Sepharose previously equilibrated with 5mM sodium phosphate buffer, pH 7, 300mM NaCl. The protein solution and the beads are mixed gently for 1 hour and poured into a column. Then, the column is washed with 5mM sodium phosphate buffer, pH 7, 300mM NaCl, until the absorbance at 280nm drops to baseline. Then, the bound protein is eluted from the column with five volumes of 5mM sodium phosphate buffer, pH 7, 700mM NaCl. Protein containing fractions are pooled and diluted with 5mM sodium phosphate buffer, pH 7 in order to lower the salt concentration to 175mM. The resulting material then is applied to the Mono Q FPLC column (Pharmacia) equilibrated with 5mM sodium phosphate buffer, pH 7 and the protein that binds to the Mono Q FPLC column (Pharmacia) is eluted as described before.

[0102] It is appreciated, however, that one skilled in the art may assess, by routine experimentation, the benefit of incorporating the second optional step into the purification

protocol. In addition, it is appreciated also that the benefit of adding each of the optional steps will depend upon the source of the starting material.

[0103] When the gp96 fraction is isolated from the 100,000g pellet, the pellet is suspended in 5 volumes of PBS containing either 1% sodium deoxycholate or 1% oxtyl glucopyranoside (but without the Mg^{2+} and Ca^{2+}) and incubated on ice for 1 hour. The suspension is centrifuged at 20,000g for 30 minutes and the resulting supernatant dialyzed against several changes of PBS (also without the Mg^{2+} and Ca^{2+}) to remove the detergent. The dialysate is centrifuged at 100,000g for 90 minutes, the supernatant harvested, and calcium and magnesium are added to the supernatant to give final concentrations of 2mM, respectively. Then the sample is purified by either the unmodified or the modified method for isolating gp96 from the 100,000g supernatant, see above.

[0104] The gp96 protein can be purified to apparent homogeneity using this procedure. About 10-20μg of gp96 can be isolated from 1g cells/tissue.

5.3.4. PREPARATION AND PURIFICATION OF α2M

[0105] Alpha-2-macroglobulin can be bought from commercial sources or prepared by purifying it from human blood.

[0106] Generally, alpha-2-macroglobulin can be recovered and purified from sera of mammals by known methods, including ammonium sulfate precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, immunoaffinity chromatography, hydroxyapatite chromatography, and lectin chromatography.

[0107] In one embodiment, α2M is purified from serum using affinity purification techniques. Methods for chromatography fractionation of proteins, such as affinity chromatography, are well known in the art. Briefly, affinity chromatography utilizes an immobilized binding partner to specifically capture the protein in the binding reaction. The binding partner molecule of the affinity capture assay can comprise, for example, an antibody to α2M or other ligand, such as an α2M receptor binding domain which specifically binds α2M. Alternatively, a filter binding assay utilizes a device, such as a solid phase surface such as a filter or a column, to non-specifically retain proteins or protein complexes based on some physical or chemical difference between the complexes and the unbound reactants. Affinity chromatography and/or filter binding separation techniques may be used to isolate α2M from serum or other bodily fluid as described herein.

[0108] In a specific embodiment of the invention, α2M is isolated from serum as follows: serum is contacted to a solid phase, such as an agarose column, which contains a binding partner of α2M, i.e., an α2M- binding molecule. The serum is allowed to incubate

on the solid phase for a period of time sufficient to allow binding of α2M with the solid phase. The material which does not bind is then removed from the solid phase; and the bound α2M is eluted from the solid phase.

[0109] The binding partner of α2M may be any molecule which specifically binds to α2M. In a preferred embodiment, the α2M - binding molecule is an antibody specific to α2M. The α2M - specific antibody is preferably a monoclonal antibody. In another preferred embodiment, the α2M - binding molecule is a ligand-binding fragment of the α2M receptor.

[0110] The solid phase may be any surface or matrix, such as, but not limited to, polycarbonate, polystyrene, polypropylene, polyethylene, glass, nitrocellulose, dextran, nylon, polyacrylamide and agarose. The support configuration can include beads, membranes, microparticles, the interior surface of a reaction vessel such as a microtiter plate, test tube or other reaction vessel.

[0111] In a preferred embodiment, α2M is isolated from serum from mice by diluting serum 1:1 with 0.04 M Tris pH 7.6, 0.15 M NaCl. The mixture is then applied to a 65ml Sephacryl S 300R (Sigma) column equilibrated and eluted with the same buffer. α2M-positive fractions are determined by dot blot and the buffer changed to a 0.01 M sodium phosphate buffer at pH 7.5 by use of a PD-10 column. Alternatively, the 0.04 M Tris pH 7.6, 0.15 M NaCl buffer can be used as buffer in the 65ml column to eliminate the step of exchanging the buffer. The complex-containing fractions are applied to a Concanavalin A sepharose column. Bound complex are eluted with 0.2M methylmannose pyranoside, or 5% methylmannose pyranoside, and applied to a DEAE column equilibrated with 0.05M sodium acetate buffer. A2M are eluted in a pure form, as analyzed by SDS-PAGE and immunoblotting with 0.13 M sodium acetate buffer.

[0112] In yet another embodiment, α2M can be isolated from blood, the following non-limiting protocol can be used by way of example: blood is collected from a subject and is allowed to clot. It is then centrifuged for 30 minutes under 14,000 x g to obtain the serum which is then applied to a gel filtration column (Sephacryl S-300R) equilibrated with 0.04M Tris buffer pH 7.6 plus 0.3M NaCl. A 65ml column is used for about 10ml of serum. Three ml fractions are collected and each fraction is tested for the presence of α2M by dot blot using an α2M specific antibody. The α2M positive fractions are pooled and applied to a PD10 column to exchange the buffer to .01M Sodium Phosphate buffer pH 7.5 with PMSF. The pooled fractions are then applied to a Con A column (10ml) equilbrated with the phosphate buffer. The column is washed and the protein is eluted with 5% methylmannose pyranoside. The eluent is passed over a PD10 column to change the buffer

to a Sodium Acetate buffer (0.05M, pH6.0). A DEAE column is then equilibrated with acetate buffer and the sample is applied to the DEAE column. The column is washed and the protein is eluted with 0.13M sodium acetate. The fractions with α2M are then pooled. The α2M can be purified to apparent homogeneity using this procedure as assayed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

[0113] Other methods for isolation of α2M known in the art can also be used (Dubin et al., 1984, Immunotherapy 8(4):589-596,; Okubo et al., 1981, Bio. Chem. Biophys. 688:257-267; Nieuwenhuizen et al. 1979, Biochem. Et Biophy. 580:129-139).

5.3.5. PREPARATION AND PURIFICATION OF HSP110

[0114] A procedure, described by Wang et al., 2001, J. Immunol. 166(1):490-7, that can be used, presented by way of example and not limitation, is as follows:

[0115] A pellet (40-60 ml) of cell or tissue, e.g., tumor cell tissue, is homogenized in 5 vol of hypotonic buffer (30 mN sodium bicarbonate, pH7.2, and protease inhibitors) by Dounce homogenization. The lysate is centrifuged at 4,500 x g and then 100,000 x g for 2 hours. If the cells or tissues are of hepatic origin, the resulting supernatant is was first applied to a blue Sepharose column (Pharmacia) to remove albumin. Otherwise, the resulting supernatant is applied to a Con A-Sepharose column (Pharmacia Biotech, Piscataway, NJ) previously equilibrated with binding buffer (20mM Tris-HCl, pH 7.5; 100mM NaCl; 1mM $MgCl_2$; 1 mM $CaCl_2$; 1 mM $MnCl_2$; and 15 mM 2-ME). The bound proteins are eluted with binding buffer containing 15% α-D-o-methylmannoside (Sigma, St. Louis, MO).

[0116] Con A-Sepharose unbound material is first dialyzed against a solution of 20 mM Tris-HCl, pH 7.5; 100 mM NaCl; and 15 mM 2-ME, and then applied to a DEAE-Sepharose column and eluted by salt gradient from 100 to 500 mM NaCl. Fractions containing hsp110 are collected, dialyzed, and loaded onto a Mono Q (Pharmacia) 10/10 column equilibrated with 20mM Tris-HCl, pH 7.5; 200 mM NaCl; and 15 mM 2-ME. The bound proteins are eluted with a 200-500 mM NaCl gradient. Fractions are analyzed by SDS-PAGE followed by immunoblotting with an Ab for hsp110, as described by Wang et al., 1999, J. Immunol. 162:3378. Pooled fractions containing hsp110 are concentrated by Centriplus (Amicon, Beverly, MA) and applied to a Superose 12 column (Pharmacia). Proteins are eluted by 40 mM Tris-HCl, pH 8.0; 150 mM NaCl; and 15 mM 2-ME with a flow rate of 0.2 ml/min.

5.3.6. PREPARATION AND PURIFICATION OF GRP170

[0117] A procedure, described by Wang et al., 2001, J. Immunol. 166(1):490-7, that can be used, presented by way of example and not limitation, is as follows:

[0118] Six A pellet (40-60 ml) of cells or tissue, e.g., tumor cell tissue, is homogenized in 5 vol of hypotonic buffer (30 mN sodium bicarbonate, pH7.2, and protease inhibitors) by Dounce homogenization. The lysate is centrifuged at 4,500 x g and then 100,000 x g for 2 hours. If the cells or tissues are of hepatic origin, the resulting supernatant is was first applied to a blue Sepharose column (Pharmacia) to remove albumin. Otherwise, the resulting supernatant is applied to a Con A-Sepharose column (Pharmacia Biotech, Piscataway, NJ) previously equilibrated with binding buffer (20mM Tris-HCl, pH 7.5; 100mM NaCl; 1mM $MgCl_2$; 1 mM $CaCl_2$; 1 mM $MnCl_2$; and 15 mM 2-ME). The bound proteins are eluted with binding buffer containing 15% α-D-o-methylmannoside (Sigma, St. Louis, MO).

[0119] Con A-Sepharose-bound material is first dialyzed against 20 mM Tris-HCl, pH 7.5, and 150 mM NaCl and then applied to a Mono Q column and eluted by a 150 to 400 mM NaCl gradient. Pooled fractions are concentrated and applied on the Superose 12 column (Pharmacia). Fractions containing homogeneous grp170 are collected.

5.3.7. RECOMBINANT EXPRESSION OF HEAT SHOCK PROTEINS AND α2M

[0120] In certain embodiments of the present invention, HSPs and α2M can be prepared from cells that express higher levels of HSPs and α2M through recombinant means. Amino acid sequences and nucleotide sequences of many HSPs and α2M are generally available in sequence databases, such as GenBank. Computer programs, such as Entrez, can be used to browse the database, and retrieve any amino acid sequence and genetic sequence data of interest by accession number. These databases can also be searched to identify sequences with various degrees of similarities to a query sequence using programs, such as FASTA and BLAST, which rank the similar sequences by alignment scores and statistics. Such nucleotide sequences of non-limiting examples of HSPs that can be used for the compositions, methods, and for preparation of the HSP peptide-complexes of the invention are as follows: human HSP70, Genbank Accession No. M24743, Hunt et al., 1995, Proc. Natl. Acad. Sci. U.S.A., 82: 6455-6489; human HSP90, Genbank Accession No. X15183, Yamazaki et al., Nucl. Acids Res. 17: 7108; human gp96: Genbank Accession No. X15187, Maki et al., 1990, Proc. Natl. Acad. Sci. U.S.A. 87: 5658-5562; human BiP: Genbank Accession No. M19645; Ting et al., 1988, DNA 7: 275-286; human HSP27, Genbank Accession No. M24743; Hickey et al., 1986, Nucleic Acids Res. 14: 4127-45; mouse HSP70: Genbank Accession No. M35021, Hunt et al., 1990, Gene 87: 199-204; mouse gp96: Genbank Accession No. M16370, Srivastava et al., 1987, Proc. Natl. Acad. Sci. U.S.A. 85: 3807-3811; and mouse BiP: Genbank Accession

No. U16274; Haas et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85: 2250-2254. Degenerate sequences encoding HSPs can also be used.

[0121] As used herein, the term "α2M" embraces other polypeptide fragments, analogs, and variants of α2M having at least 35% to 55%, preferably 55% to 75%, and most preferably 75% to 85% amino acid identity with α2M, and is capable of forming a complex with an antigenic peptide, which complex is capable of being taken up by an antigen presenting cell and eliciting an immune response against the antigenic molecule. The α2M molecule of the invention can be purchased commercially or purified from natural sources (Kurecki et al., 1979, Anal. Biochem. 99:415-420), chemically synthesized, or recombinantly produced. Non-limiting examples of α2M sequences that can be used for preparation of the α2M polypeptides of the invention are as follows: Genbank Accession Nos. M11313, P01023, AAA51551; Kan et al., 1985, Proc. Nat. Acad. Sci. 82: 2282-2286. A degenerate sequence encoding α2M can also be used.

[0122] Once the nucleotide sequence encoding the HSP or α2M of choice has been identified, the nucleotide sequence, or a fragment thereof, can be obtained and cloned into an expression vector for recombinant expression. The expression vector can then be introduced into a host cell for propagation of the HSP or α2M. Methods for recombinant production of HSPs or α2M are described in detail herein.

[0123] The DNA may be obtained by DNA amplification or molecular cloning directly from a tissue, cell culture, or cloned DNA (e.g., a DNA "library") using standard molecular biology techniques (see e.g., Methods in Enzymology, 1987, volume 154, Academic Press; Sambrook et al. 1989, Molecular Cloning - A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, New York; and Current Protocols in Molecular Biology, Ausubel et al. (eds.), Greene Publishing Associates and Wiley Interscience, New York, each of which is incorporated herein by reference in its entirety). Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the HSP or α2M gene should be cloned into a suitable vector for propagation of the gene.

[0124] In a preferred embodiment, DNA can be amplified from genomic or cDNA by polymerase chain reaction (PCR) amplification using primers designed from the known sequence of a related or homologous HSP or α2M. PCR is used to amplify the desired sequence in DNA clone or a genomic or cDNA library, prior to selection. PCR can be carried out, e.g., by use of a thermal cycler and Taq polymerase (Gene Amp®). The polymerase chain reaction (PCR) is commonly used for obtaining genes or gene fragments of interest. For example, a nucleotide sequence encoding an HSP or α2M of any desired

length can be generated using PCR primers that flank the nucleotide sequence encoding open reading fram. Alternatively, an HSP or α2M gene sequence can be cleaved at appropriate sites with restriction endonuclease(s) if such sites are available, releasing a fragment of DNA encoding the HSP or α2M gene. If convenient restriction sites are not available, they may be created in the appropriate positions by site-directed mutagenesis and/or DNA amplification methods known in the art (see, for example, Shankarappa et al., 1992, PCR Method Appl. 1: 277-278). The DNA fragment that encodes the HSP or α2M is then isolated, and ligated into an appropriate expression vector, care being taken to ensure that the proper translation reading frame is maintained.

[0125] In an alternative embodiment, for the molecular cloning of an HSP or α2M gene from genomic DNA, DNA fragments are generated to form a genomic library. Since some of the sequences encoding related HSPs or α2M are available and can be purified and labeled, the cloned DNA fragments in the genomic DNA library may be screened by nucleic acid hybridization to a labeled probe (Benton and Davis, 1977, Science 196: 180; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A. 72: 3961). Those DNA fragments with substantial homology to the probe will hybridize. It is also possible to identify an appropriate fragment by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map.

[0126] Alternatives to isolating the HSP or α2M genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or synthesizing a cDNA to the mRNA which encodes the HSP or α2M. For example, RNA for cDNA cloning of the HSP or α2M gene can be isolated from cells which express the HSP or α2M. A cDNA library may be generated by methods known in the art and screened by methods, such as those disclosed for screening a genomic DNA library. If an antibody to the HSP or α2M is available, the HSP or α2M may be identified by binding of a labeled antibody to the HSP- or α2M-synthesizing clones.

[0127] Other specific embodiments for the cloning of a nucleotide sequence encoding an HSP or α2M, are presented as examples but not by way of limitation, as follows: In a specific embodiment, nucleotide sequences encoding an HSP or α2M can be identified and obtained by hybridization with a probe comprising a nucleotide sequence encoding HSP or α2M under various conditions of stringency which are well known in the art (including those employed for cross-species hybridizations).

[0128] Any technique for mutagenesis known in the art can be used to modify individual nucleotides in a DNA sequence, for purpose of making amino acid substitution(s) in the expressed peptide sequence, or for creating/deleting restriction sites to facilitate

further manipulations. Such techniques include but are not limited to, chemical mutagenesis, in vitro site-directed mutagenesis (Hutchinson et al., 1978, J. Biol. Chem. 253: 6551), oligonucleotide-directed mutagenesis (Smith, 1985, Ann. Rev. Genet. 19: 423-463; Hill et al., 1987, Methods Enzymol. 155: 558-568), PCR-based overlap extension (Ho et al., 1989, Gene 77: 51-59), PCR-based megaprimer mutagenesis (Sarkar et al., 1990, Biotechniques 8: 404-407), etc. Modifications can be confirmed by double stranded dideoxynucleotide DNA sequencing.

[0129] In certain embodiments, a nucleic acid encoding a secretory form of a non-secreted HSP is used to practice the methods of the present invention. Such a nucleic acid can be constructed by deleting the coding sequence for the ER retention signal, KDEL. Optionally, the KDEL coding sequence is replaced with a molecular tag to facilitate the recognition and purification of the HSP, such as the Fc portion of murine IgG1. In another embodiment, a molecular tag can be added to naturally secreted HSPs or α2M. PCT publication no. WO 99/42121 demonstrates that deletion of the ER retention signal of gp96 resulted in the secretion of gp96-Ig peptide-complexes from transfected tumor cells, and the fusion of the KDEL-deleted gp96 with murine IgG1 facilitated its detection by ELISA and FACS analysis and its purification by affinity chromatography with the aid of Protein A.

5.3.7.1. EXPRESSION SYSTEMS

[0130] Nucleotide sequences encoding an HSP or α2M molecule can be inserted into the expression vector for propagation and expression in recombinant cells. An expression construct, as used herein, refers to a nucleotide sequence encoding an HSP or α2M operably associated with one or more regulatory regions which allows expression of the HSP or α2M molecule in an appropriate host cell. "Operably-associated" refers to an association in which the regulatory regions and the HSP or α2M polypeptide sequence to be expressed are joined and positioned in such a way as to permit transcription, and ultimately, translation of the HSP or α2M sequence. A variety of expression vectors may be used for the expression of HSPs or α2M, including, but not limited to, plasmids, cosmids, phage, phagemids, or modified viruses. Examples include bacteriophages such as lambda derivatives, or plasmids such as pBR322 or pUC plasmid derivatives or the Bluescript vector (Stratagene). Typically, such expression vectors comprise a functional origin of replication for propagation of the vector in an appropriate host cell, one or more restriction endonuclease sites for insertion of the HSP or α2M gene sequence, and one or more selection markers.

[0131] For expression of HSPs or α2M in mammalian host cells, a variety of regulatory regions can be used, for example, the SV40 early and late promoters, the

cytomegalovirus (CMV) immediate early promoter, and the Rous sarcoma virus long terminal repeat (RSV-LTR) promoter. Inducible promoters that may be useful in mammalian cells include but are not limited to those associated with the metallothionein II gene, mouse mammary tumor virus glucocorticoid responsive long terminal repeats (MMTV-LTR), the β-interferon gene, and the HSP70 gene (Williams et al., 1989, Cancer Res. 49: 2735-42 ; Taylor et al., 1990, Mol. Cell. Biol. 10: 165-75). The efficiency of expression of the HSP or α2M in a host cell may be enhanced by the inclusion of appropriate transcription enhancer elements in the expression vector, such as those found in SV40 virus, Hepatitis B virus, cytomegalovirus, immunoglobulin genes, metallothionein, β-actin (see Bittner et al., 1987, Methods in Enzymol. 153: 516-544; Gorman, 1990, Curr. Op. in Biotechnol. 1: 36-47).

[0132] The expression vector may also contain sequences that permit maintenance and replication of the vector in more than one type of host cell, or integration of the vector into the host chromosome. Such sequences may include but are not limited to replication origins, autonomously replicating sequences (ARS), centromere DNA, and telomere DNA. It may also be advantageous to use shuttle vectors that can be replicated and maintained in at least two types of host cells.

[0133] In addition, the expression vector may contain selectable or screenable marker genes for initially isolating or identifying host cells that contain DNA encoding an HSP or α2M. For long term, high yield production of HSPs or α2M, stable expression in mammalian cells is preferred. A number of selection systems may be used for mammalian cells, including, but not limited, to the Herpes simplex virus thymidine kinase (Wigler et al., 1977, Cell 11: 223), hypoxanthine-guanine phosphoribosyltransferase (Szybalski and Szybalski, 1962, Proc. Natl. Acad. Sci. U.S.A. 48: 2026), and adenine phosphoribosyltransferase (Lowy et al., 1980, Cell 22: 817) genes can be employed in tk^-, $hgprt^-$ or $aprt^-$ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dihydrofolate reductase (dhfr), which confers resistance to methotrexate (Wigler et al., 1980, Natl. Acad. Sci. U.S.A. 77: 3567; O'Hare et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78: 1527); gpt, which confers resistance to mycophenolic acid (Mulligan and Berg, 1981, Proc. Natl. Acad. Sci. U.S.A. 78: 2072); neomycin phosphotransferase (neo), which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., 1981, J. Mol. Biol. 150: 1); and hygromycin phosphotransferase (hyg), which confers resistance to hygromycin (Santerre et al., 1984, Gene 30: 147). Other selectable markers, such as but not limited to histidinol and Zeocin™ can also be used.

[0134] The expression construct comprising an HSP- or α2M-coding sequence operably associated with regulatory regions can be directly introduced into appropriate host cells for expression and production of the HSP or α2M complexes of the invention without further cloning (see, for example, U.S. Patent No. 5,580,859). The expression constructs may also contain DNA sequences that facilitate integration of the coding sequence into the genome of the host cell, e.g., via homologous recombination. In this instance, it is not necessary to employ an expression vector comprising a replication origin suitable for appropriate host cells in order to propagate and express the HSP or α2M molecule in the host cells.

[0135] Expression constructs containing cloned HSP or α2M coding sequences can be introduced into the mammalian host cell by a variety of techniques known in the art, including but not limited to calcium phosphate mediated transfection (Wigler et al., 1977, Cell 11: 223-232), liposome-mediated transfection (Schaefer-Ridder et al., 1982, Science 215: 166-168), electroporation (Wolff et al., 1987, Proc. Natl. Acad. Sci. 84: 3344), and microinjection (Cappechi, 1980, Cell 22: 479-488).

[0136] Any of the cloning and expression vectors described herein may be synthesized and assembled from known DNA sequences by techniques well known in the art. The regulatory regions and enhancer elements can be of a variety of origins, both natural and synthetic. Some vectors and host cells may be obtained commercially. Non-limiting examples of useful vectors are described in Appendix 5 of Current Protocols in Molecular Biology, 1988, ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience, which is incorporated herein by reference; and the catalogs of commercial suppliers such as Clontech Laboratories, Stratagene Inc., and Invitrogen, Inc.

[0137] Alternatively, number of viral-based expression systems may also be utilized with mammalian cells for recombinant expression of HSPs or α2M. Vectors using DNA virus backbones have been derived from simian virus 40 (SV40) (Hamer et al., 1979, Cell 17: 725), adenovirus (Van Doren et al., 1984, Mol. Cell Biol. 4: 1653), adeno-associated virus (McLaughlin et al., 1988, J. Virol. 62: 1963), and bovine papillomas virus (Zinn et al., 1982, Proc. Natl. Acad. Sci. 79: 4897). In cases where an adenovirus is used as an expression vector, the donor DNA sequence may be ligated to an adenovirus transcription/translation control region, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing heterologous

products in infected hosts (see, e.g., Logan and Shenk, 1984, Proc. Natl. Acad. Sci. U.S.A. 81: 3655-3659).

[0138] Bovine papillomavirus (BPV) can infect many higher vertebrates, including man, and its DNA replicates as an episome. A number of shuttle vectors have been developed for recombinant gene expression which exist as stable, multicopy (20-300 copies/cell) extrachromosomal elements in mammalian cells. Typically, these vectors contain a segment of BPV DNA (the entire genome or a 69% transforming fragment), a promoter with a broad host range, a polyadenylation signal, splice signals, a selectable marker, and "poisonless" plasmid sequences that allow the vector to be propagated in E. coli. Following construction and amplification in bacteria, the expression gene construct is transfected into cultured mammalian cells, for example, by the techniques of calcium phosphate coprecipitation or electroporation. For those host cells that do not manifest a transformed phenotype, selection of transformants is achieved by use of a dominant selectable marker, such as histidinol and G418 resistance. For example, BPV vectors such as pBCMGSNeo and pBCMGHis may be used to express HSPs or α2M (Karasuyama et al., Eur. J. Immunol. 18: 97-104; Ohe et al., Human Gene Therapy 6: 325-33) which may then be transfected into a diverse range of cell types for HSP or α2M expression.

[0139] Alternatively, the vaccinia 7.5K promoter may be used (see, e.g., Mackett et al., 1982, Proc. Natl. Acad. Sci. U.S.A. 79: 7415-7419; Mackett et al., 1984, J. Virol. 49: 857-864; Panicali et al., 1982, Proc. Natl. Acad. Sci. U.S.A. 79: 4927-4931) In cases where a human host cell is used, vectors based on the Epstein-Barr virus (EBV) origin (OriP) and EBV nuclear antigen 1 (EBNA-1; a trans-acting replication factor) may be used. Such vectors can be used with a broad range of human host cells, e.g., EBO-pCD (Spickofsky et al., 1990, DNA Prot. Eng. Tech. 2: 14-18), pDR2 and λDR2 (available from Clontech Laboratories).

[0140] Recombinant HSP or α2M expression can also be achieved by a retrovirus-based expression system. In contrast to transfection, retroviruses can efficiently infect and transfer genes to a wide range of cell types including, for example, primary hematopoietic cells. In retroviruses such as Moloney murine leukemia virus, most of the viral gene sequences can be removed and replaced with an HSP or α2M coding sequence, while the missing viral functions can be supplied in trans. The host range for infection by a retroviral vector can also be manipulated by the choice of envelope used for vector packaging.

[0141] For example, a retroviral vector can comprise a 5' long terminal repeat (LTR), a 3' LTR, a packaging signal, a bacterial origin of replication, and a selectable marker. The ND-associated antigenic peptide DNA is inserted into a position between the

5' LTR and 3' LTR, such that transcription from the 5' LTR promoter transcribes the cloned DNA. The 5' LTR comprises a promoter, including but not limited to an LTR promoter, an R region, a U5 region and a primer binding site, in that order. Nucleotide sequences of these LTR elements are well known in the art. A heterologous promoter as well as multiple drug selection markers may also be included in the expression vector to facilitate selection of infected cells (see McLauchlin et al., 1990, Prog. Nucleic Acid Res. and Molec. Biol. 38: 91-135; Morgenstern et al., 1990, Nucleic Acid Res. 18: 3587-3596; Choulika et al., 1996, J. Virol 70: 1792-1798; Boesen et al., 1994, Biotherapy 6: 291-302; Salmons and Gunzberg, 1993, Human Gene Therapy 4: 129-141; and Grossman and Wilson, 1993, Curr. Opin. in Genetics and Devel. 3: 110-114).

[0142] The recombinant cells may be cultured under standard conditions of temperature, incubation time, optical density, and media composition. Alternatively, cells may be cultured under conditions emulating the nutritional and physiological requirements of a cell in which the HSP is endogenously expressed. Modified culture conditions and media may be used to enhance production of HSP–peptide complexes. For example, recombinant cells may be grown under conditions that promote inducible HSP expression.

[0143] Alpha-2-macroglobulin and HSP polypeptides of the invention may be expressed as fusion proteins to facilitate recovery and purification from the cells in which they are expressed. For example, an HSP or α2M polypeptide may contain a signal sequence leader peptide to direct its translocation across the ER membrane for secretion into culture medium. Further, an HSP or α2M polypeptide may contain an affinity label, such as a affinity label, fused to any portion of the HSP or α2M polypeptide not involved in binding antigenic peptide, such as for example, the carboxyl terminal. The affinity label can be used to facilitate purification of the protein, by binding to an affinity partner molecule.

[0144] Various methods for production of such fusion proteins are well known in the art. The manipulations which result in their production can occur at the gene or protein level, preferably at the gene level. For example, the cloned coding region of an HSP or α2M polypeptide may be modified by any of numerous recombinant DNA methods known in the art (Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Ausubel et al., in Chapter 8 of Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, New York). It will be apparent from the following discussion that substitutions, deletions, insertions, or any combination thereof are introduced or combined to arrive at a final nucleotide sequence encoding an HSP or α2M polypeptide.

[0145] In various embodiments, fusion proteins comprising the HSP or α2M polypeptide may be made using recombinant DNA techniques. For example, a recombinant gene encoding an HSP or α2M polypeptide may be constructed by introducing an HSP or α2M gene fragment in the proper reading frame into a vector containing the sequence of an affinity label, such that the HSP or α2M polypeptide is expressed as a peptide-tagged fusion protein. Affinity labels, which may be recognized by specific binding partners, may be used for affinity purification of the HSP or α2M polypeptide.

[0146] In a preferred embodiment, the affinity label is fused at its amino terminal to the carboxyl terminal of HSP or α2M. The precise site at which the fusion is made in the carboxyl terminal is not critical. The optimal site can be determined by routine experimentation.

[0147] A variety of affinity labels known in the art may be used, such as, but not limited to, the immunoglobulin constant regions, polyhistidine sequence (Petty, 1996, Metal-chelate affinity chromatography, in Current Protocols in Molecular Biology, Vol. 2, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience), glutathione S-transferase (GST; Smith, 1993, Methods Mol. Cell Bio. 4:220-229), the E. coli maltose binding protein (Guan et al., 1987, Gene 67:21-30), and various cellulose binding domains (U.S. Patent Nos. 5,496,934; 5,202,247; 5,137,819; Tomme et al., 1994, Protein Eng. 7:117-123), etc. Other affinity labels may impart fluorescent properties to an HSP or α2M polypeptide, e.g., portions of green fluorescent protein and the like. Other possible affinity labels are short amino acid sequences to which monoclonal antibodies are available, such as but not limited to the following well known examples, the FLAG epitope, the myc epitope at amino acids 408-439, the influenza virus hemagglutinin (HA) epitope. Other affinity labels are recognized by specific binding partners and thus facilitate isolation by affinity binding to the binding partner which can be immobilized onto a solid support. Some affinity labels may afford the HSP or α2M polypeptide novel structural properties, such as the ability to form multimers. Dimerization of an HSP or α2M polypeptide with a bound peptide may increase avidity of interaction between the HSP or α2M polypeptide and its partner in the course of antigen presentation. These affinity labels are usually derived from proteins that normally exist as homopolymers. Affinity labels such as the extracellular domains of CD8 (Shiue et al., 1988, J. Exp. Med. 168:1993-2005), or CD28 (Lee et al., 1990, J. Immunol. 145:344-352), or portions of the immunoglobulin molecule containing sites for interchain disulfide bonds, could lead to the formation of multimers. As will be appreciated by those skilled in the art, many methods can be used to obtain the coding region of the above-mentioned affinity labels, including but not limited to, DNA cloning, DNA amplification,

and synthetic methods. Some of the affinity labels and reagents for their detection and isolation are available commercially.

[0148] A preferred affinity label is a non-variable portion of the immunoglobulin molecule. Typically, such portions comprise at least a functionally operative CH2 and CH3 domain of the constant region of an immunoglobulin heavy chain. Fusions are also made using the carboxyl terminus of the Fc portion of a constant domain, or a region immediately amino-terminal to the CH1 of the heavy or light chain. Suitable immunoglobulin-based affinity label may be obtained from IgG-1, -2, -3, or -4 subtypes, IgA, IgE, IgD, or IgM, but preferably IgG1. Preferably, a human immunoglobulin is used when the HSP or α2M polypeptide is intended for in vivo use for humans. Many DNA encoding immunoglobulin light or heavy chain constant regions is known or readily available from cDNA libraries. See, for example, Adams et al., Biochemistry, 1980, 19:2711-2719; Gough et al., 1980, Biochemistry, 19:2702-2710; Dolby et al., 1980, Proc. Natl. Acad. Sci. U.S.A., 77:6027-6031; Rice et al., 1982, Proc. Natl. Acad. Sci. U.S.A., 79:7862-7865; Falkner et al., 1982, Nature, 298:286-288; and Morrison et al., 1984, Ann. Rev. Immunol, 2:239-256. Because many immunological reagents and labeling systems are available for the detection of immunoglobulins, the HSP or α2M polypeptide–Ig fusion protein can readily be detected and quantified by a variety of immunological techniques known in the art, such as the use of enzyme-linked immunosorbent assay (ELISA), immunoprecipitation, fluorescence activated cell sorting (FACS), etc. Similarly, if the affinity label is an epitope with readily available antibodies, such reagents can be used with the techniques mentioned above to detect, quantitate, and isolate the HSP or α2M polypeptide containing the affinity label. In many instances, there is no need to develop specific antibodies to the HSP or α2M polypeptide.

[0149] A particularly preferred embodiment is a fusion of an HSP or α2M polypeptide to the hinge, the CH2 and CH3 domains of human immunoglobulin G-1 (IgG-1; see Bowen et al.,1996, J. Immunol. 156:442-49). This hinge region contains three cysteine residues which are normally involved in disulfide bonding with other cysteines in the Ig molecule. Since none of the cysteines are required for the peptide to function as a tag, one or more of these cysteine residues may optionally be substituted by another amino acid residue, such as for example, serine.

[0150] Various leader sequences known in the art can be used for the efficient secretion of HSP or α2M polypeptide from bacterial and mammalian cells (von Heijne, 1985, J. Mol. Biol. 184:99-105). Leader peptides are selected based on the intended host cell, and may include bacterial, yeast, viral, animal, and mammalian sequences. For example, the herpes virus glycoprotein D leader peptide is suitable for use in a variety of

mammalian cells. A preferred leader peptide for use in mammalian cells can be obtained from the V-J2-C region of the mouse immunoglobulin kappa chain (Bernard et al., 1981, Proc. Natl. Acad. Sci. 78:5812-5816). Preferred leader sequences for targeting HSP or α2M polypeptide expression in bacterial cells include, but are not limited to, the leader sequences of the E.coli proteins OmpA (Hobom et al., 1995, Dev. Biol. Stand. 84:255-262), Pho A (Oka et al., 1985, Proc. Natl. Acad. Sci 82:7212-16), OmpT (Johnson et al., 1996, Protein Expression 7:104-113), LamB and OmpF (Hoffman & Wright, 1985, Proc. Natl. Acad. Sci. USA 82:5107-5111), β-lactamase (Kadonaga et al., 1984, J. Biol. Chem. 259:2149-54), enterotoxins (Morioka-Fujimoto et al., 1991, J. Biol. Chem. 266:1728-32), and the Staphylococcus aureus protein A (Abrahmsen et al., 1986, Nucleic Acids Res. 14:7487-7500), and the B. subtilis endoglucanase (Lo et al., Appl. Environ. Microbiol. 54:2287-2292), as well as artificial and synthetic signal sequences (MacIntyre et al., 1990, Mol. Gen. Genet. 221:466-74; Kaiser et al., 1987, Science, 235:312-317).

[0151] DNA sequences encoding a desired affinity label or leader peptide, which may be readily obtained from libraries, produced synthetically, or may be available from commercial suppliers, are suitable for the practice of this invention. Such methods are well known in the art.

5.4. COMPLEXING PROTEINS AND PEPTIDES TO HSP AND α2M

[0152] Described herein are exemplary methods for complexing in vitro the HSP or α2M with an antigenic set of peptides generated from a purified target antigen as described in Section 5.2.1. In certain embodiments, the peptides are the result of digestion of a target antigen purified from antigenic cells, a cellular fraction thereof, or viral particles. In other embodiments, the peptides are the result of digestion of a synthetically or recombinantly produced target antigen. The complexing reaction can result in the formation of a covalent bond between a HSP and one or more antigenic peptides. The complexing reaction can result in the formation of a covalent bond between a α2M and one or more antigenic peptides. The complexing reaction can also result in the formation of a non-covalent association between a HSP and one or more antigenic peptides, or a α2M and one or more antigenic peptides.

[0153] Complexes can also be formed between HSP and peptides comprising more than one antigenic set of peptides. Complexes can further be formed between α2M and more than one antigenic set of peptides. In one example, HSP is complexed to two or more antigenic sets of peptides produced by treatment of a target antigen with two or more different proteases. In a specific embodiment of the invention, HSP is complexed to two

antigenic sets of peptides produced by cleavage of a target antigen with CNBr and V8 protease.

[0154] Prior to complexing, the HSPs can be pretreated with ATP or exposed to acidic conditions to remove any peptides that may be non-covalently associated with the HSP of interest. When the ATP procedure is used, excess ATP is removed from the preparation by the addition of apyranase as described by Levy, et al., 1991, Cell 67:265-274. When acidic conditions are used, the buffer is readjusted to neutral pH by the addition of pH modifying reagents. A preferred, exemplary protocol for the noncovalent complexing of a population of peptides (average length between 7 to 20 amino acids) to an HSP in vitro is discussed below:

[0155] The antigenic set of peptides (1 μg, which can be dissolved in 10% to 50% dimethyl sulfoxide) and the pretreated HSP (9 μg) are admixed to give an approximately 5 peptides (or proteins) : 1 HSP molar ratio. Then, the mixture is incubated for 15 minutes to 3 hours at 4° to 45°C in a suitable binding buffer such as phosphate buffered saline pH7.4, or one containing 20mM sodium phosphate, pH 7.2, 350mM NaCl, 3mM $MgCl_2$ and 1mM phenyl methyl sulfonyl fluoride (PMSF). The preparations are centrifuged through a Centricon 10 assembly (Millipore) to remove any unbound peptide. The non-covalent association of peptides with the HSPs can be assayed by High Performance Liquid Chromatography (HPLC) or Mass Spectrometry (MS).

[0156] An exemplary procedure for generating complexes is as follows:

[0157] Cell lysate is obtained from live Meth A cells by dounce homogenization followed by ultracentrifugation. 100,000g supernatant is treated with 0.1% trifluoroacetic acid (TFA) and 3mM ATP for 10 hours followed by centrifugation in a CENTRICON membrane filter (Millipore) with a 10kDa cut off limit. Peptides less than10 kDa (referred to as "MethA10") are further isolated by binding to a C18 reverse phase column, eluting the peptides with methanol, drying the peptides down in a vacuum, and reconstituting the peptides in a buffer suitable for complexing. Gp96, α2M, or albumin (which was used as a control) is heated to 50°C in the presence of 50 molar excess of MethA10. The reactions containing the resulting complexes are placed at room temperature for 30 minutes and then placed on ice. Free, uncomplexed peptide is removed using CENTRICON 50 (Millipore). Complexes thus made are used for immunizations.

[0158] In an alternative embodiment of the invention, preferred for complexing HSP70 non-covalently to an antigenic set of peptides, 5-10 micrograms of purified HSP70 is incubated with equimolar quantities of peptides in 20mM sodium phosphate buffer pH 7.5, 0.5M NaCl, 3mM $MgCl_2$ and 1mM ADP in a volume of 100 microliter at 37°C for 1 hr.

This incubation mixture is centrifuged one or more times if necessary, through a Centricon 10 assembly (Millipore) to remove any unbound peptide.

[0159] In an alternative embodiment of the invention, preferred for producing non-covalent complexes of gp96 or HSP90 to peptides, 5-10 micrograms of purified gp96 or HSP90 is incubated with equimolar or excess quantities of the antigenic set of peptides in a suitable buffer such as one containing 20mM sodium phosphate buffer pH 7.5, 0.5M NaCl, 3mM MgCl2 at 60-65°C for 5-20 min. This incubation mixture is allowed to cool to room temperature and centrifuged one or more times if necessary, through a Centricon 10 assembly (Millipore) to remove any unbound peptide.

[0160] Following complexing with an antigenic set of peptides, an immunogenic HSP complex or α2M complex can optionally be assayed using, for example, the mixed lymphocyte target cell assay (MLTC) described below. Once HSP-peptide complexes have been isolated and diluted, they can be optionally characterized further in animal models using the preferred administration protocols and excipients discussed below.

[0161] As an alternative to making non-covalent complexes of HSPs and peptides, an antigenic set of peptides can be covalently attached to HSPs.

[0162] In one embodiment, HSPs are covalently coupled to an antigenic set of peptides of a target antigen by chemical crosslinking. Chemical crosslinking methods are well known in the art. For example, in a preferred embodiment, glutaraldehyde crosslinking may be used. Glutaradehyde crosslinking has been used for formation of covalent complexes of peptides and HSPs (see Barrios et al., 1992, Eur. J. Immunol. 22: 1365-1372). Preferably, 1-2 mg of HSP-peptide complex is crosslinked in the presence of 0.002% glutaraldehyde for 2 hours. Glutaraldehyde is removed by dialysis against phosphate buffered saline (PBS) overnight (Lussow et al., 1991, Eur. J. Immunol. 21: 2297-2302). Alternatively, a HSP and an antigenic set of peptides can be crosslinked by ultraviolet (UV) crosslinking under conditions known in the art.

[0163] In another embodiment of the invention, an antigenic set of peptides can be non-covalently complexed to α2M by incubating the proteins/peptides with α2M at a 50:1 molar ratio and incubated at 50° C for 10 minutes followed by a 30 minute incubation at 25°C. Free (uncomplexed) peptides can be removed by size exclusion filters. Complexes are preferably measured by a scintillation counter to make sure that on a per molar basis, each HSP or α2M is observed to bind equivalent amounts of peptide (approximately 0.1% of the starting amount of the antigenic set of peptides). For details, see Binder, 2001, J. Immunol. 166(8):4968-72, which is incorporated herein by reference in its entirety. To reduce the propensity of forming covalent complexes of α2M and the peptides in these

reactions, it will be desirable to inhibit or remove protease activity prior to complexing. This can be accomplished with the use of protease inhibitors, for example, by the methods described in section 4.2.1. Also desirable is adding a reducing agent (such as 2-mercaptoethanol) to the reactions to neutralize nucleophilic compounds present in the target antigen which may activate α2M for covalent association.

[0164] In yet another embodiment, an antigenic set of peptides can be complexed to α2M covalently by methods as described in PCT publications WO 94/14976 and WO 99/50303 for complexing a peptide to α2M, which are incorporated herein by reference in their entirety. For example, an antigenic set of peptides can be incorporated into α2M by ammonia or methylamine (or other small amine nucleophiles such as ethylamine) during reversal of the nucleophilic activation, employing heat (Grøn and Pizzo, 1998, Biochemistry, 37: 6009-6014; which is incorporated herein by reference in its entirety). Such conditions that allow fortuitous trapping of peptides by α2M can be employed to prepare the α2M complexes of the invention. Covalent linking of peptides to α2M can also be performed using a bifunctional crosslinking agent. Such crosslinking agents and methods of their use are also well known in the art. Preferably, the crosslinking agent is inactivated and/or removed after the complexes are formed. Methods for covalent coupling have been described previously (Osada et al., 1987, Biochem. Biophys. Res. Commun.146:26-31; Osada et al., 1988, Biochem. Biophys. Res. Commun. 150:883; Chu and Pizzo, 1993, J. Immunol. 150:48; Chu et al., 1994, Ann. N.Y. Acad. Sci. 737:291-307; Mitsuda et al., 1993, Biochem. Biophys. Res. Commun. 101:1326-1331).

[0165] In yet another embodiment, an antigenic set of peptides can be complexed to a mixture of different HSPs in the same reaction by the non-covalent or covalent methods described above.

[0166] In yet another embodiment, an antigenic set of peptides can be complexed to a mixture of one or more HSP and α2M in the same reaction by the non-covalent or covalent methods described above.

[0167] Complexes of a single type of HSP and one or more different antigenic sets of peptides from separate covalent and/or non-covalent complexing reactions can optionally be combined to form a composition before administration to a subject. Complexes of different HSPs and one or more different antigenic sets of peptides from separate covalent and/or non-covalent complexing reactions can optionally be combined to form a composition before administration to a subject. Complexes of α2M and one or more different antigenic sets of peptides from separate covalent and/or non-covalent complexing reactions can also optionally be combined to form a composition before administration to a

subject. Complexes comprising one or more type of HSP and α2M, and one or more different antigenic sets of peptides from separate covalent and/or non-covalent complexing reactions can optionally be combined to form a composition before administration to a subject.

5.5. PREVENTION AND TREATMENT OF CANCER AND INFECTIOUS DISEASES

[0168] In accordance with the invention, a composition of the invention, which comprises an antigenic set of peptides and a HSP and/or α2M, is administered to a subject with cancer or an infectious disease. In one embodiment, "treatment" or "treating" refers to an amelioration of cancer or an infectious disease, or at least one discernible symptom thereof. In another embodiment, "treatment" or "treating" refers to an amelioration of at least one measurable physical parameter associated with cancer or an infectious disease, not necessarily discernible by the subject. In yet another embodiment, "treatment" or "treating" refers to inhibiting the progression of a cancer or an infectious disease, which can be either physically, e.g., stabilization of a discernible symptom, physiologically, e.g., stabilization of a physical parameter, or both.

[0169] In certain embodiments, the compositions of the present invention are administered to a subject as a preventative measure against such cancer or an infectious disease. As used herein, "prevention" or "preventing" refers to a reduction of the risk of acquiring a given cancer or infectious disease. In the context of cancer, the compositions of the present invention can be used to decrease the formation of precancerous or cancer cells, to inhibit the growth or transformation of cancer or pre-cancerous cells, to inhibit or decrease metastasis, or to modify or reduce the malignant phenotypes of cancer or pre-cancerous cells. In one mode of the embodiment, the compositions of the present invention are administered as a preventative measure to a subject having a genetic predisposition to a cancer. In another mode of the embodiment, the compositions of the present invention are administered as a preventive measure to a subject facing exposure to carcinogens including but not limited to chemicals and/or radiation, or to a subject facing exposure to an agent of an infectious disease.

[0170] For example, in certain embodiments, administration of the compositions of the invention leads to an inhibition or reduction of the growth of cancerous cells or infectious agents by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 45%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the growth in absence of said composition.

[0171] The compositions prepared by methods of the invention comprise complexes of heat shock protein(s) with an antigenic set of peptides, and/or complexes of alpha-2-macroglobulin with an antigenic set of peptides. The compositions appear to induce an inflammatory reaction towards the tumor at at the tumor site, and can ultimately cause a regression of the tumor burden in the cancer patients treated. The compositions prepared by the methods of the invention can enhance the immunocompetence of the subject and elicit specific immunity against infectious agents, specific immunity against preneoplastic and neoplastic cells, or specific immunity against antigens that are associated with a metabolic disorder (e.g., cardiovascular, neurological, hormonal disorders). These compositions have the capacity to prevent the onset and progression of infectious diseases, to inhibit the growth and progression of tumor cells, and the onset and progression of certain disorders (e.g., cardiovascular, neurological, metabolic, hormonal disorders).

[0172] Combination therapy refers to the use of HSP complexes or α2M complexes of the invention with another modality to prevent or treat cancer and infectious diseases. As used herein, a vaccine therapeutic modality is a modality in which it is desired to induce/increase an immune response for therapeutic efficacy. The administration of the complexes of the invention can augment the effect of anti-cancer agents or anti-infectives, and vice versa. Preferably, this additional form of modality is a non-HSP and non-α2M based modality, i.e., this modality does not comprise either HSP or α2M as a component. This approach is commonly termed combination therapy, adjunctive therapy or conjunctive therapy (the terms are used interchangeably herein). With combination therapy, additive potency or additive therapeutic effect can be observed. Synergistic outcomes where the therapeutic efficacy is greater than additive can also be expected. The use of combination therapy can also provide better therapeutic profiles than the administration of the treatment modality, or the HSP complexes or α2M complexes alone. The additive or synergistic effect may allow the dosage and/or dosing frequency of either or both modalities be adjusted to reduce or avoid unwanted or adverse effects.

[0173] In various specific embodiments, the combination therapy comprises the administration of HSP complexes or α2M complexes to a subject treated with a treatment modality wherein the treatment modality administered alone is not clinically adequate to treat the subject such that the subject needs additional effective therapy, e.g., a subject is unresponsive to a treatment modality without administering HSP complexes or α2M complexes. Included in such embodiments are methods comprising administering HSP complexes or α2M complexes to a subject receiving a treatment modality wherein said subject has responded to therapy yet suffers from side effects, relapse, develops resistance,

etc. Such a subject might be non-responsive or refractory to treatment with the treatment modality alone, i.e., at least some significant portion of cancer cells or pathogens are not killed or their cell division is not arrested. The embodiments provide that the methods of the invention comprising administration of HSP complexes to a subject refractory to a treatment modality alone can improve the therapeutic effectiveness of the treatment modality when administered as contemplated by the methods of the invention. The methods of the invention comprising administration of an α2M complexes to a subject refractory to a treatment modality alone can also improve the therapeutic effectiveness of the treatment modality when administered as contemplated by the methods of the invention. The determination of the effectiveness of a treatment modality can be assayed in vivo or in vitro using methods known in the art. Art-accepted meanings of refractory are well known in the context of cancer. In one embodiment, a cancer or infectious disease is refractory or non-responsive where respectively, the number of cancer cells or pathogens has not been significantly reduced, or has increased. Among these subjects being treated are those receiving chemotherapy or radiation therapy.

[0174] According to the invention, complexes of the invention can be used in combination with many different types of treatment modalities. Some of such modalities are particularly useful for a specific type of cancer or infectious disease and are discussed in Section 5.5.1 and 5.5.2. Many other modalities have an effect on the functioning of the immune system and are applicable generally to both neoplastic and infectious diseases.

[0175] In one embodiment, complexes of the invention are used in combination with one or more biological response modifiers to treat cancer or infectious disease. One group of biological response modifiers is the cytokines. In one such embodiment, a cytokine is administered to a subject receiving HSP/α2M complexes. In another such embodiment, HSP/α2M complexes are administered to a subject receiving a chemotherapeutic agent in combination with a cytokine. In various embodiments, one or more cytokine(s) can be used and are selected from the group consisting of IL-1α, IL- 1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFNα, IFNβ, IFNγ, TNFα, TNFβ, G-CSF, GM-CSF, TGF-β, IL-15, IL-18, GM-CSF, INF-γ, INF-α, SLC, endothelial monocyte activating protein-2 (EMAP2), MIP-3α, MIP-3β, or an MHC gene, such as HLA-B7. Addtionally, other exemplary cytokines include other members of the TNF family, including but not limited to TNF-α-related apoptosis-inducing ligand (TRAIL), TNF-α-related activation-induced cytokine (TRANCE), TNF-α-related weak inducer of apoptosis (TWEAK), CD40 ligand (CD40L), lymphotoxin alpha (LT-α), lymphotoxin beta (LT-β), OX40, Fas ligand (FasL), CD27 ligand (CD27L), CD30 ligand (CD30L), 41BB, APRIL, LIGHT, TL1,

TNFSF10, TNFSF17, and AITR-L, or a functional portion thereof. See, e.g., Kwon et al., 1999, Curr. Opin. Immunol. 11:340-345 for a general review of the TNF family. Preferably, the HSP complexes or α2M complexes are administered prior to the treatment modalities. In a specific embodiment, complexes of the invention are administered to a subject receiving cyclophosphamide in combination with IL-12 for treatment of cancer.

[0176] In another embodiments, complexes of the invention are used in combination with one or more biological response modifiers which are agonists or antagonists of various ligands, receptors and signal transduction molecules of the immune system. For examples, the biological response modifiers include but are not limited to agoinsts of Toll-like receptors (TLR-2, TLR-7, TLR-8 and TLR-9; LPS; agonists of 41BB ligand, OX40 ligand, ICOS, and CD40; and antagonists of Fas ligand, PD1, and CTLA-4. These agonists and antagonists can be antibodies, antibody fragments, peptides, peptidomimetic compounds, and polysaccharides.

[0177] In yet another embodiment, complexes of the invention are used in combination with one or more biological response modifiers which are immunostimulatory nucleic acids. Such nucleic acids, many of which are oligonucleotides comprising an unmethylated CpG motif, are mitogenic to vertebrate lymphocytes, and are known to enhance the immune response. See Woolridge, et al., 1997, Blood 89:2994-2998. Such oligonucleotides are described in International Patent Publication Nos. WO 01/22972, WO 01/51083, WO 98/40100 and WO 99/61056, each of which is incorporated herein in its entirety, as well as United States Patent Nos. 6,207,646, 6,194,388, 6,218,371, 6,239,116, 6,429,199, and 6,406,705, each of which is incorporated herein in its entirety. Other kinds of immunostimulatory oligonucleotides such as phosphorothioate oligodeoxynucleotides containing YpG- and CpR-motifs have been described by Kandimalla et al. in "Effect of Chemical Modifications of Cytosine and Guanine in a CpG-Motif of Oligonucleotides: Structure-Immunostimulatory Activity Relationships." Bioorganic & Medicinal Chemistry 9:807-813 (2001), incorporated herein by reference in its entirety. Also encompassed are immunostimulatory oligonucleotides that lack CpG dinucleotides which when administered by mucosal routes (including low dose administration) or at high doses through parenteral routes, augment antibody responses, often as much as did the CpG nucleic acids, however the response was Th2-biased (IgG1>>IgG2a). See United States Patent Publication No. 20010044416 A1, which is incorporated herein by reference in its entirety. Methods of determining the activity of immunostimulatory oligonucleotides can be performed as described in the aforementioned patents and publications. Moreover, immunostimulatory oligonucleotides can be modified within the phosphate backbone, sugar,

nucleobase and internucleotide linkages in order to modulate the activity. Such modifications are known to those of skill in the art.

[0178] In yet another embodiment, complexes of the invention are used in combination with one or more adjuvants. The adjuvant(s) can be administered separately or present in a composition in admixture with complexes of the invention. A systemic adjuvant is an adjuvant that can be delivered parenterally. Systemic adjuvants include adjuvants that creates a depot effect, adjuvants that stimulate the immune system and adjuvants that do both. An adjuvant that creates a depot effect as used herein is an adjuvant that causes the antigen to be slowly released in the body, thus prolonging the exposure of immune cells to the antigen. This class of adjuvants includes but is not limited to alum (e.g., aluminum hydroxide, aluminum phosphate); or emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720, AirLiquide, Paris, France); MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, Calif.; and PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micelle-forming agent; IDEC, Pharmaceuticals Corporation, San Diego, Calif.).

[0179] Other adjuvants stimulate the immune system, for instance, cause an immune cell to produce and secrete cytokines or IgG. This class of adjuvants includes but is not limited to immunostimulatory nucleic acids, such as CpG oligonucleotides; saponins purified from the bark of the South American Quillaja saponaria tree; poly[di(carboxylatophen- oxy)phosphazene (PCPP polymer; Virus Research Institute, USA); derivatives of lipopolysaccharides (LPS) such as monophosphoryl lipid A (MPL; Ribi ImmunoChem Research, Inc., Hamilton, Mont.), muramyl dipeptide (MDP; Ribi) andthreonyl-muramyl dipeptide (t-MDP; Ribi); OM-174 (a glucosamine disaccharide related to lipid A; OM Pharma SA, Meyrin, Switzerland); and Leishmania elongation factor (a purified Leishmania protein; Corixa Corporation, Seattle, Wash.).

[0180] Saponins include glycosidic triterpenoid compounds commonly purified from an aqueous extract of the bark of Quillaja saponaria Molina. Saponins, and chemically modified saponins, are described in U.S. Patent Nos. 5,057,540, and 5,443,829, both of which are herein expressly incorporated by reference in their entireties. Representative saponins useful in the methods and compositions of the present invention include, but are not limited to, QS-7, QS-17, QS-18, and QS-21, also known as QA-7, QA-17, QA-18, and QA-21, respectively, and the component fractions of QS-21, i.e., QA-21-V1 and QA-21-V2.

[0181] Other systemic adjuvants are adjuvants that create a depot effect and stimulate the immune system. These compounds are those compounds which have both of the above-identified functions of systemic adjuvants. This class of adjuvants includes but is not limited to ISCOMs (Immunostimulating complexes which contain mixed saponins, lipids and form virus-sized particles with pores that can hold antigen; CSL, Melbourne, Australia); SB-AS2 (SmithKline Beecham adjuvant system #2 which is an oil-in-water emulsion containing MPL and QS21: SmithKline Beecham Biologicals [SBB], Rixensart, Belgium); SB-AS4 (SmithKline Beecham adjuvant system #4 which contains alum and MPL; SBB, Belgium); non-ionic block copolymers that form micelles such as CRL 1005 (these contain a linear chain of hydrophobic polyoxpropylene flanked by chains of polyoxyethylene; Vaxcel, Inc., Norcross, Ga.); and Syntex Adjuvant Formulation (SAF, an oil-in-water emulsion containing Tween 80 and a nonionic block copolymer; Syntex Chemicals, Inc., Boulder, Colo.).

[0182] The mucosal adjuvants useful according to the invention are adjuvants that are capable of inducing a mucosal immune response in a subject when administered to a mucosal surface in conjunction with complexes of the invention. Mucosal adjuvants include but are not limited to CpG nucleic acids (e.g. PCT published patent application WO 99/61056), Bacterial toxins: e.g., Cholera toxin (CT), CT derivatives including but not limited to CT B subunit (CTB) (Wu et al., 1998, Tochikubo et al., 1998); CTD53 (Val to Asp) (Fontana et al., 1995); CTK97 (Val to Lys) (Fontana et al., 1995); CTK104 (Tyr to Lys) (Fontana et al., 1995); CTD53/K63 (Val to Asp, Ser to Lys) (Fontana et al., 1995); CTH54 (Arg to His) (Fontana et al., 1995); CTN107 (His to Asn) (Fontana et al., 1995); CTE114 (Ser to Glu) (Fontana et al., 1995); CTE112K (Glu to Lys) (Yamamoto et al., 1997a); CTS61F (Ser to Phe) (Yamamoto et al., 1997a, 1997b); CTS106 (Pro to Lys) (Douce et al., 1997, Fontana et al., 1995); and CTK63 (Ser to Lys) (Douce et al., 1997, Fontana et al., 1995), Zonula occludens toxin, zot, Escherichia coli heat-labile enterotoxin, Labile Toxin (LT), LT derivatives including but not limited to LT B subunit (LTB) (Verweij et al., 1998); LT7K (Arg to Lys) (Komase et al., 1998, Douce et al., 1995); LT61F (Ser to Phe) (Komase et al., 1998); LT112K (Glu to Lys) (Komase et al., 1998); LT118E (Gly to Glu) (Komase et al., 1998); LT146E (Arg to Glu) (Komase et al., 1998); LT192G (Arg to Gly) (Komase et al., 1998); LTK63 (Ser to Lys) (Marchetti et al., 1998, Douce et al., 1997, 1998, Di Tommaso et al., 1996); and LTR72 (Ala to Arg) (Giuliani et al., 1998), Pertussis toxin, PT. (Lycke et al., 1992, Spangler BD, 1992, Freytag and Clemments, 1999, Roberts et al., 1995, Wilson et al., 1995) including PT-9K/129G (Roberts et al., 1995, Cropley et al., 1995); Toxin derivatives (see below) (Holmgren et al., 1993, Verweij et al.,

1998; Kappuon et al., 1995, Freytag and Clements, 1999); Lipid A derivatives (e.g., monophosphoryl lipid A, MPL) (Sasaki et al., 1998, Vancott et al., 1998; Muramyl Dipeptide (MDP) derivatives (Fukushima et al., 1996, Ogawa et al., 1989, Michalek et al., 1983, Morisaki et al., 1983); bacterial outer membrane proteins (e.g., outer surface protein A (OspA) lipoprotein of Borrelia burgdorferi, outer membrane protine of Neisseria meningitidis)(Marinaro et al., 1999, Van de Verg et al., 1996); oil-in-water emulsions (e.g., MF59) (Barchfield et al., 1999, Verschoor et al., 1999, O'Hagan, 1998); aluminum salts (Isaka et al., 1998, 1999); and Saponins (e.g., QS21) Aquila Biopharmaceuticals, Inc., Worster, Me.) (Sasaki et al., 1998, MacNeal et al., 1998), ISCOMs, MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, Calif.); the Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720; AirLiquide, Paris, France); PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micell-forming agent; IDEC Pharmaceuticals Corporation, San Diego, Calif.); Syntext Adjuvant Formulation (SAF; Syntex Chemicals, Inc., Boulder, Colo.); poly[di(carboxylatophenoxy)phosphazene (PCPP polymer; Virus Research Institute, USA) and Leishmania elongation factor (Corixa Corporation, Seattle, Wash.).

[0183] In a preferred embodiment, the complexes of the invention are administered in combination with QS-21, ODNs, α-CTLA-4, α-4-1BB, CEL-1000, RC-529 (AGPs), MPL, a TLR agonist, IL-12, α-Galactosyl-ceramide, Imiquimod/Resiquimid, GM-CSF, LPS, [1-3] beta-D-glucan, IL-1, Muramyl dipeptide, Muramyl tripeptide phosphatidylethanolamine, copolymer, MF-5, SAF, Quil-A, polyphosphazene, NISV, BCG stress protein, and/or SRL 172.

[0184] In certain embodiments that encompass treatment and prevention of metabolic disorders other than cancer and infectious diseases, the compositions of the invention are administered in combination with one or more other therapeutic modalities to treat or prevent these disorders. Nonlimiting examples of therapeutic modalities that may be administered in combination with the compositions of the invention for the treatment and prevention of neurological disorder such as Alzheimer's disease or dementia include cholinesterase inhibitors, e.g., donepezil (Aricept®); rivastigmine (Exelon®); and galantamine (Reminyl®); Tacrine (Cognex®). N-methyl-D-aspartate (NMDA) receptor antagonist, e.g., Memantine (Namenda®). Vitamin E; Medications that can control depression, anxiety, and psychotic symptoms; NSAIDs (non-steroidal anti-inflammatory drugs).

[0185] Nonlimiting examples of therapeutic modalities that may be administered in combination with the composition of the invention for the treatment and prevention of

asthma include; ADVAIR, Anticholinergics (Atrovent®, Combivent®), Beta-Agonists (Albuterol, Alupent® (metaproterenol), Maxair® and Maxair Autohaler® (pirbuterol), Proventil®, Proventil HFA®, and Ventolin® (albuterol), Xopenex® (levalbuterol)); Steroids (Deltasone® (prednisone), Medrol® (methylprednisolone), Orapred®, Prelone® , Pediapred® (prednisolone)).

[0186] Nonlimiting examples of therapeutic modalities that may be administered in combination with a composition of the invention for the treatment and prevention of hypertension include diuretics (Acetazolamide (Diamox®), Indapamide (Lozol®), Metolazone (Zaroxolyn®), Spirnolactone (Aldactone®), Torsemide (Demadex®), Triamterene (Dyrenium®)); Beta blockers (Atenolol (Tenormin®), Bisoprolol (Zebeta®), Carvedilol (Coreg®), Metoprolol (iLopressor®, Toprol SL®), Timolol (Blockadren®)); Calcium channel blockers (diltiazem (Cardizem®) and verapamil (Calan®, Covera HS®, Isoptin®, Veralan®), Amlodipine (Norvasc®), Felodipine (Plendil®), Idradipine (DynaCirc®), Nicardipine (Cardene®), Nisoldipine (Sular®));ACE inhibitors (Benazepril (Lotensin®), Captopril (Capoten®), Enalapril (Vasotec®), Fosinopril (Monopril®), Lisinopril (Prinivil®, Zestril®), Quinapril (Accupril®), Ramipril (Altace®), Trandolapril (Mavik®));Angiotensin-receptor blockers ();vasodilators, e.g., hydralazine; clonidine (Catapres®) and methyldopa (Aldomet®).

[0187] Nonlimiting examples of therapeutic modalities that may be administered in combination with a composition of the invention for the treatment and prevention of atherosclerosis include bile acid sequestrants (e.g., cholestyramine, colestipol, and colesevelam), fibric acids (e.g., gemfibrozil, fenofibrate), nicotinic acid, and statins (e.g., lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, and cerivastatin).

5.5.1. TARGET CANCERS

[0188] In one embodiment, combination therapy encompasses, in addition to the administration of the complexes of the invention, the adjunctive uses of one or more modalities that aid in the prevention or treatment of cancer, which modalities include, but is not limited to chemotherapeutic agents, immunotherapeutics, anti-angiogenic agents, cytokines, hormones, antibodies, polynucleotides, radiation and photodynamic therapeutic agents. In specific embodiments, combination therapy can be used to prevent the recurrence of cancer, inhibit metastasis, or inhibit the growth and/or spread of cancer or metastasis.

[0189] Types of cancers that can be treated or prevented by the methods of the present invention include, but are not limited to human sarcomas and carcinomas, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma,

angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease.

[0190] Preferably, the cancers to be treated by the methods and compositions of the invention include renal cell carcinoma, melanoma, lung cancer, breast cancer, lymphoma, leukemia, colorectal cancer and pancreatic cancer.

[0191] In another embodiment, the patient having a cancer is immunosuppressed by reason of having undergone anti-cancer therapy (e.g., chemotherapy radiation) prior to administration of the HSP and/or α2M-peptide complexes or administration of the HSP- and/or α2M- sensitized APC.

[0192] There are many reasons why immunotherapy as provided by the present invention is desired for use in cancer patients. First, surgery with anesthesia may lead to immunosuppression. With appropriate immunotherapy in the preoperative period, this immunosuppression may be prevented or reversed. This could lead to fewer infectious complications and to accelerated wound healing. Second, tumor bulk is minimal following surgery and immunotherapy is most likely to be effective in this situation. A third reason is the possibility that tumor cells are shed into the circulation at surgery and effective immunotherapy applied at this time can eliminate these cells.

[0193] The preventive and therapeutic methods of the invention are directed at enhancing the immunocompetence of the cancer patient either before surgery, at or after surgery, and to induce tumor-specific immunity to cancer cells, with the objective being inhibition of cancer, and with the ultimate clinical objective being total cancer regression

and eradication. The methods of the invention can also be used in individuals at enhanced risk of a particular type of cancer, e.g., due to familial history or environmental risk factors.

[0194] In various embodiments, one or more anti-cancer agent, in addition to the complexes of the invention, is administered to treat a cancer patient. An anti-cancer agent refers to any molecule or compound that assists in the treatment of tumors or cancer. Examples of anti-cancer agents that may be used in the methods of the present invention include, but are not limited to: acivicin; aclarubicin; acodazole hydrochloride; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone acetate; aminoglutethimide; amsacrine; anastrozole; anthramycin; asparaginase; asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa; bicalutamide; bisantrene hydrochloride; bisnafide dimesylate; bizelesin; bleomycin sulfate; brequinar sodium; bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer; carboplatin; carmustine; carubicin hydrochloride; carzelesin; cedefingol; chlorambucil; cirolemycin; cisplatin; cladribine; crisnatol mesylate; cyclophosphamide; cytarabine; dacarbazine; dactinomycin; daunorubicin hydrochloride; decitabine; dexormaplatin; dezaguanine; dezaguanine mesylate; diaziquone; docetaxel; doxorubicin; doxorubicin hydrochloride; droloxifene; droloxifene citrate; dromostanolone propionate; duazomycin; edatrexate; eflornithine hydrochloride; elsamitrucin; enloplatin; enpromate; epipropidine; epirubicin hydrochloride; erbulozole; esorubicin hydrochloride; estramustine; estramustine phosphate sodium; etanidazole; etoposide; etoposide phosphate; etoprine; fadrozole hydrochloride; fazarabine; fenretinide; floxuridine; fludarabine phosphate; fluorouracil; flurocitabine; fosquidone; fostriecin sodium; gemcitabine; gemcitabine hydrochloride; hydroxyurea; idarubicin hydrochloride; ifosfamide; ilmofosine; interleukin II (including recombinant interleukin II, or rIL2), interferon alfa-2a; interferon alfa-2b; interferon alfa-n1 ; interferon alfa-n3; interferon beta-I a; interferon gamma-I b; iproplatin; irinotecan hydrochloride; lanreotide acetate; letrozole; leuprolide acetate; liarozole hydrochloride; lometrexol sodium; lomustine; losoxantrone hydrochloride; masoprocol; maytansine; mechlorethamine hydrochloride; megestrol acetate; melengestrol acetate; melphalan; menogaril; mercaptopurine; methotrexate; methotrexate sodium; metoprine; meturedepa; mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin; mitosper; mitotane; mitoxantrone hydrochloride; mycophenolic acid; nocodazole; nogalamycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin; pentamustine; peplomycin sulfate; perfosfamide; pipobroman; piposulfan; piroxantrone hydrochloride; plicamycin; plomestane; porfimer sodium; porfiromycin; prednimustine; procarbazine hydrochloride; puromycin; puromycin hydrochloride; pyrazofurin; riboprine; rogletimide; safingol;

saffingol hydrochloride; semustine; simtrazene; sparfosate sodium; sparsomycin; spirogermanium hydrochloride; spiromustine; spiroplatin; streptonigrin; streptozocin; sulofenur; talisomycin; tecogalan sodium; tegafur; teloxantrone hydrochloride; temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine; thiotepa; tiazofurin; tirapazamine; toremifene citrate; trestolone acetate; triciribine phosphate; trimetrexate; trimetrexate glucuronate; triptorelin; tubulozole hydrochloride; uracil mustard; uredepa; vapreotide; verteporfin; vinblastine sulfate; vincristine sulfate; vindesine; vindesine sulfate; vinepidine sulfate; vinglycinate sulfate; vinleurosine sulfate; vinorelbine tartrate; vinrosidine sulfate; vinzolidine sulfate; vorozole; zeniplatin; zinostatin; zorubicin hydrochloride.

[0195] Other anti-cancer drugs that can be used include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; antiandrogen, prostatic carcinoma; antiestrogen; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin 1; axinastatin 2; axinastatin 3; azasetron; azatoxin; azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstaurosporine; beta lactam derivatives; beta-alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors (ICOS); castanospermine; cecropin B; cetrorelix; chlorlns; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; cladribine; clomifene analogues; clotrimazole; collismycin A; collismycin B; combretastatin A4; combretastatin analogue; conagenin; crambescidin 816; crisnatol; cryptophycin 8; cryptophycin A derivatives; curacin A; cyclopentanthraquinones; cycloplatam; cypemycin; cytarabine ocfosfate; cytolytic factor; cytostatin; dacliximab; decitabine; dehydrodidemnin B; deslorelin; dexamethasone; dexifosfamide; dexrazoxane; dexverapamil; diaziquone; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol, 9-; dioxamycin; diphenyl spiromustine; docetaxel; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab;

eflornithine; elemene; enediyne; epirubicin; epristeride; estramustine analogue; estrogen agonists; estrogen antagonists; etanidazole; etoposide phosphate; exemestane; fadrozole; fazarabine; fenretinide; filgrastim; finasteride; flavopiridol; flezelastine; fluasterone; fludarabine; fluorodaunorunicin hydrochloride; forfenimex; formestane; fostriecin; fotemustine; gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase inhibitors; gemcitabine; glutathione inhibitors; hepsulfam; heregulin; hexamethylene bisacetamide; hypericin; ibandronic acid; idarubicin; idoxifene; idramantone; ilmofosine; ilomastat; imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth factor-1 receptor inhibitor; interferon agonists; interferons; interleukins; iobenguane; iododoxorubicin; ipomeanol, 4-; iroplact; irsogladine; isobengazole; isohomohalicondrin B; itasetron; jasplakinolide; kahalalide F; lamellarin-N triacetate; lanreotide; leinamycin; lenograstim; lentinan sulfate; leptolstatin; letrozole; leukemia inhibiting factor; leukocyte alpha interferon; leuprolide+estrogen+progesterone; leuprorelin; levamisole; liarozole; linear polyamine analogue; lipophilic disaccharide peptide; lipophilic platinum compounds; lissoclinamide 7; lobaplatin; lombricine; lometrexol; lonidamine; losoxantrone; lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; lytic peptides; maitansine; mannostatin A; marimastat; masoprocol; maspin; matrilysin inhibitors; matrix metalloproteinase inhibitors; menogaril; merbarone; meterelin; methioninase; metoclopramide; MIF inhibitor; mifepristone; miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, human chorionic gonadotrophin; monophosphoryl lipid A+myobacterium cell wall sk; mopidamol; multiple drug resistance gene inhibitor; multiple tumor suppressor 1-based therapy; mustard anti-cancer agent; mycaperoxide B; mycobacterial cell wall extract; myriaporone; N-acetyldinaline; N-substituted benzamides; nafarelin; nagrestip; naloxone+pentazocine; napavin; naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide antioxidant; nitrullyn; O6-benzylguanine; octreotide; okicenone; oligonucleotides; onapristone; ondansetron; ondansetron; oracin; oral cytokine inducer; ormaplatin; osaterone; oxaliplatin; oxaunomycin; paclitaxel; paclitaxel analogues; paclitaxel derivatives; palauamine; palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin; pazelliptine; pegaspargase; peldesine; pentosan polysulfate sodium; pentostatin; pentrozole; perflubron; perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum complex; platinum compounds;

platinum-triamine complex; porfimer sodium; porfiromycin; prednisone; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C inhibitors, microalgal; protein tyrosine phosphatase inhibitors; purine nucleoside phosphorylase inhibitors; purpurins; pyrazoloacridine; pyridoxylated hemoglobin polyoxyethylene conjugate; raf antagonists; raltitrexed; ramosetron; ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitor; retelliptine demethylated; rhenium Re 186 etidronate; rhizoxin; ribozymes; RII retinamide; rogletimide; rohitukine; romurtide; roquinimex; rubiginone B1; ruboxyl; safingol; saintopin; SarCNU; sarcophytol A; sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen binding protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; telomerase inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; tirapazamine; titanocene bichloride; topsentin; toremifene; totipotent stem cell factor; translation inhibitors; tretinoin; triacetyluridine; triciribine; trimetrexate; triptorelin; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; UBC inhibitors; ubenimex; urogenital sinus-derived growth inhibitory factor; urokinase receptor antagonists; vapreotide; variolin B; vector system, erythrocyte gene therapy; velaresol; veramine; verdins; verteporfin; vinorelbine; vinxaltine; vitaxin; vorozole; zanoterone; zeniplatin; zilascorb; and zinostatin stimalamer.

[0196] An anti-cancer agent can be a chemotherapeutic agents which include but are not limited to, the following groups of compounds : cytotoxic antibiotics, antimetabolities, anti-mitotic agents, alkylating agents, platinum compounds, arsenic compounds, DNA topoisomerase inhibitors, taxanes, nucleoside analogues, plant alkaloids, and toxins; and synthetic derivatives thereof. Table 1 lists exemplary compounds of the groups:

TABLE 1

Alkylating agents	
Nitrogen mustards:	Cyclophosphamide

	Ifosfamide
	Trofosfamide
	Chlorambucil
Nitrosoureas:	Carmustine (BCNU)
	Lomustine (CCNU)
Alkylsulphonates:	Busulfan
	Treosulfan
Triazenes:	Dacarbazine
Platinum containing compounds:	Cisplatin
	Carboplatin
	Aroplatin
	Oxaliplatin
Plant Alkaloids	
Vinca alkaloids:	Vincristine
	Vinblastine
	Vindesine
	Vinorelbine
Taxoids:	Paclitaxel
	Docetaxol
DNA Topoisomerase Inhibitors	
Epipodophyllins:	Etoposide
	Teniposide
	Topotecan
	9-aminocamptothecin
	Camptothecin
	Crisnatol
mitomycins:	Mitomycin C
Anti-folates:	
DHFR inhibitors:	Methotrexate
	Trimetrexate
IMP dehydrogenase Inhibitors:	Mycophenolic acid
	Tiazofurin
	Ribavirin
	EICAR
Ribonuclotide reductase Inhibitors:	Hydroxyurea
	Deferoxamine
Pyrimidine analogs:	
Uracil analogs:	5-Fluorouracil
	Floxuridine
	Doxifluridine
	Ratitrexed
Cytosine analogs:	Cytarabine (ara C)
	Cytosine arabinoside
	Fludarabine
Purine analogs:	Mercaptopurine
	Thioguanine
DNA Antimetabolites:	3-HP
	2'-deoxy-5-fluorouridine
	5-HP

	alpha-TGDR
	aphidicolin glycinate
	ara-C
	5-aza-2'-deoxycytidine
	beta-TGDR
	cyclocytidine
	guanazole
	inosine glycodialdehyde
	macebecin II
	pyrazoloimidazole
Antimitotic agents:	allocolchicine
	Halichondrin B
	colchicine
	colchicine derivative
	dolstatin 10
	maytansine
	rhizoxin
	thiocolchicine
	trityl cysteine
Others:	
Isoprenylation inhibitors:	
Dopaminergic neurotoxins:	1-methyl-4-phenylpyridinium ion
Cell cycle inhibitors:	Staurosporine
Actinomycins:	Actinomycin D
	Dactinomycin
Bleomycins:	Bleomycin A2
	Bleomycin B2
	Peplomycin
Anthracyclines:	Daunorubicin
	Doxorubicin (adriamycin)
	Idarubicin
	Epirubicin
	Pirarubicin
	Zorubicin
	Mitoxantrone
MDR inhibitors:	Verapamil
Ca^{2+}ATPase inhibitors:	Thapsigargin

[0197] Compositions comprising one or more chemotherapeutic agents (e.g., FLAG, CHOP) are also contemplated by the present invention. FLAG comprises fludarabine, cytosine arabinoside (Ara-C) and G-CSF. CHOP comprises cyclophosphamide, vincristine, doxorubicin, and prednisone. Each of the foregoing lists is illustrative, and is not intended to be limiting.

[0198] Preferably, the following therapeutic agents are used in combination with the compositions of the invention to prevent or treat various cancers: anti-CTLA4, anti-CD20 (Rituxan), Herceptin (anti-HER2), Avastin (anti-VEGF, vascular endothelial growth factor), Erbitux (anti-epidermal growth factor receptor (EGFR)), deferoxamine, ATRA,

cyclophosphamide, interferon-alpha, anti-41BB, anti-CD25, angiostatin, endostatin, thalidomide, endotoxins (e.g., LPS, MPL), celpene, tyrosine kinase inhibitiors (e.g. BAY 43-9006, tarceva), resiquimod, talabostat, ontak, 1-methyl tryptophan, IL-18, IL-21, and/or velcade.

[0199] In one embodiment, breast cancer can be treated with a pharmaceutical composition comprising complexes of the invention in combination with 5-fluorouracil, cisplatin, docetaxel, doxorubicin, Herceptin®, gemcitabine, IL-2, paclitaxel, and/or VP-16 (etoposide).

[0200] In another embodiment, prostate cancer can be treated with a pharmaceutical composition comprising complexes of the invention in combination with paclitaxel, docetaxel, mitoxantrone, and/or an androgen receptor antagonist (e.g., flutamide).

[0201] In another embodiment, leukemia can be treated with a pharmaceutical composition comprising complexes of the invention in combination with fludarabine, cytosine arabinoside, gemtuzumab (MYLOTARG), daunorubicin, methotrexate, vincristine, 6-mercaptopurine, idarubicin, mitoxantrone, etoposide, asparaginase, prednisone and/or cyclophosphamide. As another example, myeloma can be treated with a pharmaceutical composition comprising complexes of the invention in combination with dexamethasone. Preferably, the leukemia is chronic myeloid leukemia (CML), the HSP complexes comprises hsp70-peptide complexes, and the therapeutic modality is imatinib mesylate or Gleevec™.

[0202] In another embodiment, melanoma can be treated with a pharmaceutical composition comprising complexes of the invention in combination with dacarbazine.

[0203] In another embodiment, colorectal cancer can be treated with a pharmaceutical composition comprising complexes of the invention in combination with irinotecan.

[0204] In another embodiment, lung cancer can be treated with a pharmaceutical composition comprising complexes of the invention in combination with paclitaxel, docetaxel, etoposide and/or cisplatin.

[0205] In another embodiment, non-Hodgkin's lymphoma can be treated with a pharmaceutical composition comprising complexes of the invention in combination with cyclophosphamide, CHOP, etoposide, bleomycin, mitoxantrone and/or cisplatin.

[0206] In another embodiment, gastric cancer can be treated with a pharmaceutical composition comprising complexes of the invention in combination with cisplatin.

[0207] In another embodiment, pancreatic cancer can be treated with a pharmaceutical composition comprising complexes of the invention in combination with gemcitabine.

[0208] According to the invention, the complexes of the invention can be administered prior to, subsequently, or concurrently with anti-cancer agent(s), for the prevention or treatment of cancer. Depending on the type of cancer, the subject's history and condition, and the anti-cancer agent(s) of choice, the use of the complexes of the invention can be coordinated with the dosage and timing of chemotherapy.

[0209] The use of the complexes of the invention can be added to a regimen of chemotherapy. In one embodiment, the chemotherapeutic agent is gemcitabine at a dose ranging from 100 to 1000 mg/m^2/cycle. In one embodiment, the chemotherapeutic agent is dacarbazine at a dose ranging from 200 to 4000 mg/m^2/cycle. In a preferred embodiment, the dose of dacarbazine ranges from 700 to 1000 mg/m^2/cycle. In another embodiment, the chemotherapeutic agent is fludarabine at a dose ranging from 25 to 50 mg/m^2/cycle. In another embodiment, the chemotherapeutic agent is cytosine arabinoside (Ara-C) at a dose ranging from 200 to 2000 mg/m^2/cycle. In another embodiment, the chemotherapeutic agent is docetaxel at a dose ranging from 1.5 to 7.5 mg/kg/cycle. In another embodiment, the chemotherapeutic agent is paclitaxel at a dose ranging from 5 to 15 mg/kg/cycle. In yet another embodiment, the chemotherapeutic agent is cisplatin at a dose ranging from 5 to 20 mg/kg/cycle. In yet another embodiment, the chemotherapeutic agent is 5-fluorouracil at a dose ranging from 5 to 20 mg/kg/cycle. In yet another embodiment, the chemotherapeutic agent is doxorubicin at a dose ranging from 2 to 8 mg/kg/cycle. In yet another embodiment, the chemotherapeutic agent is epipodophyllotoxin at a dose ranging from 40 to 160 mg/kg/cycle. In yet another embodiment, the chemotherapeutic agent is cyclophosphamide at a dose ranging from 50 to 200 mg/kg/cycle. In yet another embodiment, the chemotherapeutic agent is irinotecan at a dose ranging from 50 to 75, 75 to 100, 100 to 125, or 125 to 150 mg/m^2/cycle. In yet another embodiment, the chemotherapeutic agent is vinblastine at a dose ranging from 3.7 to 5.4, 5.5 to 7.4, 7.5 to 11, or 11 to 18.5 mg/m^2/cycle. In yet another embodiment, the chemotherapeutic agent is vincristine at a dose ranging from 0.7 to 1.4, or 1.5 to 2 mg/m^2/cycle. In yet another embodiment, the chemotherapeutic agent is methotrexate at a dose ranging from 3.3 to 5, 5 to 10, 10 to 100, or 100 to 1000 mg/m^2/cycle.

[0210] In a preferred embodiment, the invention further encompasses the use of low doses of chemotherapeutic agents when administered as part of the combination therapy regimen. For example, initial treatment with the complexes of the invention increases the

sensitivity of a tumor to subsequent challenge with a dose of chemotherapeutic agent, which dose is near or below the lower range of dosages when the chemotherapeutic agent is administered without complexes of the invention.

[0211] In one embodiment, complexes of the invention and a low dose (e.g., 6 to 60 mg/m^2/day or less) of docetaxel are administered to a cancer patient. In another embodiment, complexes of the invention and a low dose (e.g., 10 to 135 mg/m^2/day or less) of paclitaxel are administered to a cancer patient. In yet another embodiment, complexes of the invention and a low dose (e.g., 2.5 to 25 mg/m^2/day or less) of fludarabine are administered to a cancer patient. In yet another embodiment, complexes of the invention and a low dose (e.g., 0.5 to 1.5 g/m^2/day or less) of cytosine arabinoside (Ara-C) are administered to a cancer patient. In another embodiment, the chemotherapeutic agent is gemcitabine at a dose ranging from 10 to 100mg/m^2/cycle. In another embodiment, the chemotherapeutic agent is cisplatin, e.g., PLATINOL or PLATINOL-AQ (Bristol Myers), at a dose ranging from 5 to 10, 10 to 20, 20 to 40, or 40 to 75 mg/m^2/cycle. In yet another embodiment, a dose of cisplatin ranging from 7.5 to 75 mg/m^2/cycle is administered to a patient with ovarian cancer. In yet another embodiment, a dose of cisplatin ranging from 5 to 50 mg/m^2/cycle is administered to a patient with bladder cancer. In yet another embodiment, the chemotherapeutic agent is carboplatin, e.g., PARAPLATIN (Bristol Myers), at a dose ranging from 2 to 4, 4 to 8, 8 to 16, 16 to 35, or 35 to 75 mg/m^2/cycle. In yet another embodiment, a dose of carboplatin ranging from 7.5 to 75 mg/m^2/cycle is administered to a patient with ovarian cancer. In another embodiment, a dose of carboplatin ranging from 5 to 50 mg/m^2/cycle is administered to a patient with bladder cancer. In yet another embodiment, a dose of carboplatin ranging from 2 to 20 mg/m^2/cycle is administered to a patient with testicular cancer. In yet another embodiment, the chemotherapeutic agent is docetaxel, e.g., TAXOTERE (Rhone Poulenc Rorer) at a dose ranging from 6 to 10, 10 to 30, or 30 to 60 mg/m^2/cycle. In yet another embodiment, the chemotherapeutic agent is paclitaxel, e.g., TAXOL (Bristol Myers Squibb), at a dose ranging from 10 to 20, 20 to 40, 40 to 70, or 70 to 135 mg/kg/cycle. In yet another embodiment, the chemotherapeutic agent is 5-fluorouracil at a dose ranging from 0.5 to 5 mg/kg/cycle. In yet another embodiment, the chemotherapeutic agent is doxorubicin, e.g., ADRIAMYCIN (Pharmacia & Upjohn), DOXIL (Alza), RUBEX (Bristol Myers Squibb), at a dose ranging from 2 to 4, 4 to 8, 8 to 15, 15 to 30, or 30 to 60 mg/kg/cycle.

[0212] In another embodiment, complexes of the invention are administered in combination with one or more immunotherapeutic agents, such as antibodies and vaccines. In a preferred embodiment, the antibodies have in vivo therapeutic and/or prophylactic uses

against cancer. In some embodiments, the antibodies can be used for treatment and/or prevention of infectious disease. Examples of therapeutic and prophylactic antibodies include, but are not limited to, MDX-010 (Medarex, NJ) which is a humanized anti-CTLA-4 antibody currently in clinic for the treatment of prostate cancer; SYNAGIS® (MedImmune, MD) which is a humanized anti-respiratory syncytial virus (RSV) monoclonal antibody for the treatment of patients with RSV infection; HERCEPTIN® (Trastuzumab) (Genentech, CA) which is a humanized anti-HER2 monoclonal antibody for the treatment of patients with metastatic breast cancer. Other examples are a humanized anti-CD18 $F(ab')_2$ (Genentech); CDP860 which is a humanized anti-CD18 $F(ab')_2$ (Celltech, UK); PRO542 which is an anti-HIV gp120 antibody fused with CD4 (Progenics/Genzyme Transgenics); Ostavir which is a human anti Hepatitis B virus antibody (Protein Design Lab/Novartis); PROTOVIR™ which is a humanized anti-CMV IgG1 antibody (Protein Design Lab/Novartis); MAK-195 (SEGARD) which is a murine anti-TNF-α $F(ab')_2$ (Knoll Pharma/BASF); IC14 which is an anti-CD14 antibody (ICOS Pharm); a humanized anti-VEGF IgG1 antibody (Genentech); OVAREX™ which is a murine anti-CA 125 antibody (Altarex); PANOREX™ which is a murine anti-17-IA cell surface antigen IgG2a antibody (Glaxo Wellcome/Centocor); BEC2 which is a murine anti-idiotype (GD3 epitope) IgG antibody (ImClone System); IMC-C225 which is a chimeric anti-EGFR IgG antibody (ImClone System); VITAXIN™ which is a humanized anti-$\alpha V\beta 3$ integrin antibody (Applied Molecular Evolution/MedImmune); Campath 1H/LDP-03 which is a humanized anti CD52 IgG1 antibody (Leukosite); Smart M195 which is a humanized anti-CD33 IgG antibody (Protein Design Lab/Kanebo); RITUXAN™ which is a chimeric anti-CD20 IgG1 antibody (IDEC Pharm/Genentech, Roche/Zettyaku); LYMPHOCIDE™ which is a humanized anti-CD22 IgG antibody (Immunomedics); Smart ID10 which is a humanized anti-HLA antibody (Protein Design Lab); ONCOLYM™ (Lym-1) is a radiolabelled murine anti-HLA DIAGNOSTIC REAGENT antibody (Techniclone); ABX-IL8 is a human anti-IL8 antibody (Abgenix); anti-CD11a is a humanized IgG1 antibody (Genentech/Xoma); ICM3 is a humanized anti-ICAM3 antibody (ICOS Pharm); IDEC-114 is a primatized anti-CD80 antibody (IDEC Pharm/Mitsubishi); ZEVALIN™ is a radiolabelled murine anti-CD20 antibody (IDEC/Schering AG); IDEC-131 is a humanized anti-CD40L antibody (IDEC/Eisai); IDEC-151 is a primatized anti-CD4 antibody (IDEC); IDEC-152 is a primatized anti-CD23 antibody (IDEC/Seikagaku); SMART anti-CD3 is a humanized anti-CD3 IgG (Protein Design Lab); 5G1.1 is a humanized anti-complement factor 5 (C5) antibody (Alexion Pharm); D2E7 is a humanized anti-TNF-α antibody (CAT/BASF); CDP870 is a humanized anti-TNF-α Fab fragment (Celltech); IDEC-151 is a

primatized anti-CD4 IgG1 antibody (IDEC Pharm/SmithKline Beecham); MDX-CD4 is a human anti-CD4 IgG antibody (Medarex/Eisai/Genmab); CDP571 is a humanized anti-TNF-α IgG4 antibody (Celltech); LDP-02 is a humanized anti-α4β7 antibody (LeukoSite/Genentech); OrthoClone OKT4A is a humanized anti-CD4 IgG antibody (Ortho Biotech); ANTOVA™ is a humanized anti-CD40L IgG antibody (Biogen); ANTEGREN™ is a humanized anti-VLA-4 IgG antibody (Elan); MDX-33 is a human anti-CD64 (FcγR) antibody (Medarex/Centeon); SCH55700 is a humanized anti-IL-5 IgG4 antibody (Celltech/Schering); SB-240563 and SB-240683 are humanized anti-IL-5 and IL-4 antibodies, respectively, (SmithKline Beecham); rhuMab-E25 is a humanized anti-IgE IgG1 antibody (Genentech/Norvartis/Tanox Biosystems); ABX-CBL is a murine anti CD-147 IgM antibody (Abgenix); BTI-322 is a rat anti-CD2 IgG antibody (Medimmune/Bio Transplant); Orthoclone/OKT3 is a murine anti-CD3 IgG2a antibody (ortho Biotech); SIMULECT™ is a chimeric anti-CD25 IgG1 antibody (Novartis Pharm); LDP-01 is a humanized anti-β_2-integrin IgG antibody (LeukoSite); Anti-LFA-1 is a murine anti CD18 F(ab')$_2$ (Pasteur-Merieux/Immunotech); CAT-152 is a human anti-TGF-β_2 antibody (Cambridge Ab Tech); and Corsevin M is a chimeric anti-Factor VII antibody (Centocor). The above-listed immunoreactive reagents, as well as any other immunoreactive reagents, may be administered according to any regimen known to those of skill in the art, including the regimens recommended by the suppliers of the immunoreactive reagents.

[0213] In a preferred embodiment, complexes of the invention are administered in combination with IFNα, IL-2, Dacarbazine (Bayer), Temozolomide (Schering), Tamoxifen (AZ), Carmustine (BMS), Melphalan (GSK), Procarbazine (Sigma-Tau), Vinblastine, carboplatin, cisplatin, taxol, cyclophosphamide, doxorubin, Rituxan (Genentech/Roche), Herceptin (Genentech/Roche), Gleevec, Iressa (AZ), Avastin (Genentech/Roche), or Tarceva (Genentech/Roche).

[0214] In another embodiment, complexes of the invention is administered in combination with one or more anti-angiogenic agents, which includes, but is not limited to, angiostatin, thalidomide, kringle 5, endostatin, Serpin (Serine Protease Inhibitor) anti-thrombin, 29 kDa N–terminal and a 40 kDa C-terminal proteolytic fragments of fibronectin, 16 kDa proteolytic fragment of prolactin, 7.8 kDa proteolytic fragment of platelet factor-4 , a 13-amino acid peptide corresponding to a fragment of platelet factor-4 (Maione et al., 1990, Cancer Res. 51:2077-2083), a 14-amino acid peptide corresponding to a fragment of collagen I (Tolma et al., 1993, J. Cell Biol. 122:497-511), a 19 amino acid peptide corresponding to a fragment of Thrombospondin I (Tolsma et al., 1993, J. Cell Biol. 122:497-511), a 20-amino acid peptide corresponding to a fragment of SPARC (Sage et al.,

1995, J. Cell. Biochem. 57:1329-1334), or any fragments, family members, or variants thereof, including pharmaceutically acceptable salts thereof.

[0215] Other peptides that inhibit angiogenesis and correspond to fragments of laminin, fibronectin, procollagen, and EGF have also been described (see, e.g., Cao, 1998, Prog Mol Subcell Biol. 20:161-176). Monoclonal antibodies and cyclic pentapeptides, which block certain integrins that bind RGD proteins (i.e., possess the peptide motif Arg-Gly-Asp), have been demonstrated to have anti-vascularization activities (Brooks et al., 1994, Science 264:569-571; Hammes et al., 1996, Nature Medicine 2:529-533). Moreover, inhibition of the urokinase plasminogen activator receptor by receptor antagonists inhibits angiogenesis, tumor growth and metastasis (Min et al., 1996, Cancer Res. 56: 2428-33; Crowley et al., 1993, Proc Natl Acad Sci. 90:5021-25). Use of such anti-angiogenic agents in combination with the complexes is also contemplated by the present invention.

[0216] In yet another embodiment, complexes of the invention is used in association with a hormonal treatment. Hormonal therapeutic treatments comprise hormonal agonists, hormonal antagonists (e.g., flutamide, bicalutamide, tamoxifen, raloxifene, leuprolide acetate (LUPRON), LH-RH antagonists), inhibitors of hormone biosynthesis and processing, and steroids (e.g., dexamethasone, retinoids, deltoids, betamethasone, cortisol, cortisone, prednisone, dehydrotestosterone, glucocorticoids, mineralocorticoids, estrogen, testosterone, progestins), vitamin A derivatives (e.g., all-trans retinoic acid (ATRA)); vitamin D3 analogs; antigestagens (e.g., mifepristone, onapristone), and antiandrogens (e.g., cyproterone acetate).

[0217] In yet another embodiment, complexes of the invention are used in association with a gene therapy program in the treatment of cancer. In one embodiment, gene therapy with recombinant cells secreting interleukin-2 is administered in combination with complexes of the invention to prevent or treat cancer, particularly breast cancer (See, e.g., Deshmukh et al., 2001, J Neurosurg. 94:287-92). In other embodiments, gene therapy is conducted with the use of polynucleotide compounds, such as but not limited to antisense polynucleotides, ribozymes, RNA interference molecules, triple helix polynucleotides and the like, where the nucleotide sequence of such compounds are related to the nucleotide sequences of DNA and/or RNA of genes that are linked to the initiation, progression, and/or pathology of a tumor or cancer. For example, many are oncogenes, growth factor genes, growth factor receptor genes, cell cycle genes, DNA repair genes, and are well known in the art.

[0218] In another embodiment, complexes of the invention are administered in conjunction with a regimen of radiation therapy. For radiation treatment, the radiation can

be gamma rays or X-rays. The methods encompass treatment of cancer comprising radiation therapy, such as external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, palladium, iridium), radioisotopes such as strontium-89, thoracic radiation therapy, intraperitoneal P-32 radiation therapy, and/or total abdominal and pelvic radiation therapy. For a general overview of radiation therapy, see Hellman, Chapter 16: Principles of Cancer Management: Radiation Therapy, 6th edition, 2001, DeVita et al., eds., J.B. Lippencott Company, Philadelphia. In preferred embodiments, the radiation treatment is administered as external beam radiation or teletherapy wherein the radiation is directed from a remote source. In various preferred embodiments, the radiation treatment is administered as internal therapy or brachytherapy wherein a radiaoactive source is placed inside the body close to cancer cells or a tumor mass. Also encompassed is the combined use of complexes of the invention with photodynamic therapy comprising the administration of photosensitizers, such as hematoporphyrin and its derivatives, Vertoporfin (BPD-MA), phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A; and 2BA-2-DMHA.

[0219] In various embodiments, complexes of the invention are administered, in combination with at least one chemotherapeutic agent, for a short treatment cycle to a cancer patient to treat cancer. The duration of treatment with the chemotherapeutic agent may vary according to the particular cancer therapeutic agent used. The invention also contemplates discontinuous administration or daily doses divided into several partial administrations. An appropriate treatment time for a particular cancer therapeutic agent will be appreciated by the skilled artisan, and the invention contemplates the continued assessment of optimal treatment schedules for each cancer therapeutic agent. The present invention contemplates at least one cycle, preferably more than one cycle during which a single therapeutic or sequence of therapeutics is administered. An appropriate period of time for one cycle will be appreciated by the skilled artisan, as will the total number of cycles, and the interval between cycles.

[0220] In another embodiment, complexes of the invention are used in combination with compounds that ameliorate the symptoms of the cancer (such as but not limited to pain) and the side effects produced by the complexes of the invention (such as but not limited to flu-like symptoms, fever, etc). Accordingly, many compounds known to reduce pain, flu-like symptoms, and fever can be used in combination or in admixture with complexes of the invention. Such compounds include analgesics (e.g, acetaminophen), decongestants (e.g., pseudoephedrine), antihistamines (e.g., chlorpheniramine maleate), and cough suppressants (e.g., dextromethorphan).

5.5.2. TARGET INFECTIOUS DISEASES

[0221] Infectious diseases that can be treated or prevented by the methods of the present invention are caused by infectious agents including, but not limited to, viruses, bacteria, fungi protozoa, helminths, and parasites. The invention is not limited to treating or preventing infectious diseases caused by intracellular pathogens. Many medically relevant microorganisms have been described extensively in the literature, e.g., see C.G.A Thomas, Medical Microbiology, Bailliere Tindall, Great Britain 1983, the entire contents of which is hereby incorporated by reference.

[0222] In preferred embodiments, the compositions and methods of the invention can be used to prevent or treat infectious diseases caused by the following: hepatitis viruses (e.g., HBV, HCV), human immunodeficiency virus (HIVs), papilloma viruses (e.g., HPV), herpes viruses (e.g., HSV); Mycobacterium tuberculosis, influenza viruses, Bacillus anthracis, Staphylococcus aureus, Heliobacter pylori, Streptococcus species, Plasmodium falciparum, Leishmania parasites.

[0223] Combination therapy encompasses in addition to the administration of complexes of the invention, the uses of one or more modalities that aid in the prevention or treatment of infectious diseases, which modalities include, but is not limited to antibiotics, antivirals, antiprotozoal compounds, antifungal compounds, and antihelminthics. Other treatment modalities that can be used to treat or prevent infectious diseases include immunotherapeutics, polynucleotides, antibodies, cytokines, and hormones as described above.

[0224] Infectious diseases that can be treated by the methods of the invention include viral dieases of both human and non-human vertebrates, including infections by retroviruses, RNA viruses and DNA viruses. Examples of virus that have been found in humans include but are not limited to: Retroviridae (e.g. human immunodeficiency viruses, such as HIV-1 (also referred to as HTLV-III, LAV or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; Picornaviridae (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (e.g. strains that cause gastroenteritis); Togaviridae (e.g. equine encephalitis viruses, rubella viruses); Flaviridae (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g. coronaviruses); Rhabdoviridae (e.g. vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g. ebola viruses); Paramyxoviridae (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g. influenza viruses); Bungaviridae (e.g. Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); Arena viridae (hemorrhagic fever viruses); Reoviridae (e.g. reoviruses, orbiviurses and rotaviruses); Birnaviridae; Hepadnaviridae (Hepatitis B virus); Parvovirida (parvoviruses); Papovaviridae

(papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g. African swine fever virus); and unclassified viruses (e.g. the etiological agents of Spongiform encephalopathies, the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B hepatitis (class 1=internally transmitted; class 2=parenterally transmitted (i.e. Hepatitis C); Norwalk and related viruses, and astroviruses).

[0225] Diseases caused by retroviruses that are contemplated include diseases by both simple retroviruses and complex retroviruses. The simple retroviruses include the subgroups of B-type retroviruses, C-type retroviruses and D-type retroviruses. An example of a B-type retrovirus is mouse mammary tumor virus (MMTV). The C-type retroviruses include subgroups C-type group A (including Rous sarcoma virus (RSV), avian leukemia virus (ALV), and avian myeloblastosis virus (AMV)) and C-type group B (including murine leukemia virus (MLV), feline leukemia virus (FeLV), murine sarcoma virus (MSV), gibbon ape leukemia virus (GALV), spleen necrosis virus (SNV), reticuloendotheliosis virus (RV) and simian sarcoma virus (SSV)). The D-type retroviruses include Mason-Pfizer monkey virus (MPMV) and simian retrovirus type 1 (SRV-1). The complex retroviruses include the subgroups of lentiviruses, T-cell leukemia viruses and the foamy viruses. Lentiviruses include HIV-1, but also include HIV-2, SIV, Visna virus, feline immunodeficiency virus (FIV), and equine infectious anemia virus (EIAV). The T-cell leukemia viruses include HTLV-1, HTLV-II, simian T-cell leukemia virus (STLV), and bovine leukemia virus (BLV). The foamy viruses include human foamy virus (HFV), simian foamy virus (SFV) and bovine foamy virus (BFV).

[0226] Examples of RNA viruses that cause diseases in vertebrate animals include, but are not limited to, the following: members of the family Reoviridae, including the genus Orthoreovirus (multiple serotypes of both mammalian and avian retroviruses), the genus Orbivirus (Bluetongue virus, Eugenangee virus, Kemerovo virus, African horse sickness virus, and Colorado Tick Fever virus), the genus Rotavirus (human rotavirus, Nebraska calf diarrhea virus, murine rotavirus, simian rotavirus, bovine or ovine rotavirus, avian rotavirus); the family Picornaviridae, including the genus Enterovirus (poliovirus, Coxsackie virus A and B, enteric cytopathic human orphan (ECHO) viruses, hepatitis A virus, Simian enteroviruses, Murine encephalomyelitis (ME) viruses, Poliovirus muris, Bovine enteroviruses, Porcine enteroviruses, the genus Cardiovirus (Encephalomyocarditis virus (EMC), Mengovirus), the genus Rhinovirus (Human rhinoviruses including at least

1T3 subtypes; other rhinoviruses), the genus Apthovirus (Foot and Mouth disease (FMDV); the family Calciviridae, including Vesicular exanthema of swine virus, San Miguel sea lion virus, Feline picornavirus and Norwalk virus; the family Togaviridae, including the genus Alphavirus (Eastern equine encephalitis virus, Semliki forest virus, Sindbis virus, Chikungunya virus, O'Nyong-Nyong virus, Ross river virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus), the genus Flavirius (Mosquito borne yellow fever virus, Dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Kunjin virus, Central European tick borne virus, Far Eastern tick borne virus, Kyasanur forest virus, Louping III virus, Powassan virus, Omsk hemorrhagic fever virus), the genus Rubivirus (Rubella virus), the genus Pestivirus (Mucosal disease virus, Hog cholera virus, Border disease virus); the family Bunyaviridae, including the genus Bunyvirus (Bunyamwera and related viruses, California encephalitis group viruses), the genus Phlebovirus (Sandfly fever Sicilian virus, Rift Valley fever virus), the genus Nairovirus (Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus), and the genus Uukuvirus (Uukuniemi and related viruses); the family Orthomyxoviridae, including the genus Influenza virus (Influenza virus type A, many human subtypes); Swine influenza virus, and Avian and Equine Influenza viruses; influenza type B (many human subtypes), and influenza type C (possible separate genus); the family paramyxoviridae, including the genus Paramyxovirus (Parainfluenza virus type 1, Sendai virus, Hemadsorption virus, Parainfluenza viruses types 2 to 5, Newcastle Disease Virus, Mumps virus), the genus Morbillivirus (Measles virus, subacute sclerosing panencephalitis virus, distemper virus, Rinderpest virus), the genus Pneumovirus (respiratory syncytial virus (RSV), Bovine respiratory syncytial virus and Pneumonia virus of mice); forest virus, Sindbis virus, Chikungunya virus, O'Nyong-Nyong virus, Ross river virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus), the genus Flavirius (Mosquito borne yellow fever virus, Dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus, Murray Valley encephalitis virus, West Nile virus, Kunjin virus, Central European tick borne virus, Far Eastern tick borne virus, Kyasanur forest virus, Louping III virus, Powassan virus, Omsk hemorrhagic fever virus), the genus Rubivirus (Rubella virus), the genus Pestivirus (Mucosal disease virus, Hog cholera virus, Border disease virus); the family Bunyaviridae, including the genus Bunyvirus (Bunyamwera and related viruses, California encephalitis group viruses), the genus Phlebovirus (Sandfly fever Sicilian virus, Rift Valley fever virus), the genus Nairovirus (Crimean-Congo hemorrhagic fever virus, Nairobi sheep disease virus), and the genus Uukuvirus (Uukuniemi and related viruses); the family Orthomyxoviridae, including the genus Influenza virus (Influenza virus

type A, many human subtypes), Swine influenza virus, and Avian and Equine influenza viruses; influenza type B (many human subtypes), and influenza type C (possible separate genus); the family paramyxoviridae, including the genus Paramyxovirus (Parainfluenza virus type 1, Sendai virus, Hemadsorption virus, Parainfluenza viruses types 2 to 5, Newcastle Disease Virus, Mumps virus), the genus Morbillivirus (Measles virus, subacute sclerosing panencephalitis virus, distemper virus, Rinderpest virus), the genus Pneumovirus (respiratory syncytial virus (RSV), Bovine respiratory syncytial virus and Pneumonia virus of mice); the family Rhabdoviridae, including the genus Vesiculovirus (VSV), Chandipura virus, Flanders-Hart Park virus), the genus Lyssavirus (Rabies virus), fish Rhabdoviruses, and two probable Rhabdoviruses (Marburg virus and Ebola virus); the family Arenaviridae, including Lymphocytic choriomeningitis virus (LCM), Tacaribe virus complex, and Lassa virus; the family Coronoaviridae, including Infectious Bronchitis Virus (IBV), Mouse Hepatitis virus, Human enteric corona virus, and Feline infectious peritonitis (Feline coronavirus).

[0227] Illustrative DNA viruses that cause diseases in vertebrate animals include, but are not limited to: the family Poxviridae, including the genus Orthopoxvirus (Variola major, Variola minor, Monkey pox Vaccinia, Cowpox, Buffalopox, Rabbitpox, Ectromelia), the genus Leporipoxvirus (Myxoma, Fibroma), the genus Avipoxvirus (Fowlpox, other avian poxvirus), the genus Capripoxvirus (sheeppox, goatpox), the genus Suipoxvirus (Swinepox), the genus Parapoxvirus (contagious postular dermatitis virus, pseudocowpox, bovine papular stomatitis virus); the family Iridoviridae (African swine fever virus, Frog viruses 2 and 3, Lymphocystis virus of fish); the family Herpesviridae, including the alpha-Herpesviruses (Herpes Simplex Types 1 and 2, Varicella-Zoster, Equine abortion virus, Equine herpes virus 2 and 3, pseudorabies virus, infectious bovine keratoconjunctivitis virus, infectious bovine rhinotracheitis virus, feline rhinotracheitis virus, infectious laryngotracheitis virus) the Beta-herpesviruses (Human cytomegalovirus and cytomegaloviruses of swine, monkeys and rodents); the gamma-herpesviruses (Epstein-Barr virus (EBV), Marek's disease virus, Herpes saimiri, Herpesvirus ateles, Herpesvirus sylvilagus, guinea pig herpes virus, Lucke tumor virus); the family Adenoviridae, including the genus Mastadenovirus (Human subgroups A,B,C,D,E and ungrouped; simian adenoviruses (at least 23 serotypes), infectious canine hepatitis, and adenoviruses of cattle, pigs, sheep, frogs and many other species, the genus Aviadenovirus (Avian adenoviruses); and non-cultivatable adenoviruses; the family Papoviridae, including the genus Papillomavirus (Human papilloma viruses, bovine papilloma viruses, Shope rabbit papilloma virus, and various pathogenic papilloma viruses of other species), the genus

Polyomavirus (polyomavirus, Simian vacuolating agent (SV-40), Rabbit vacuolating agent (RKV), K virus, BK virus, JC virus, and other primate polyoma viruses such as Lymphotrophic papilloma virus); the family Parvoviridae including the genus Adeno-associated viruses, the genus Parvovirus (Feline panleukopenia virus, bovine parvovirus, canine parvovirus, Aleutian mink disease virus, etc). Finally, DNA viruses may include viruses which do not fit into the above families such as Kuru and Creutzfeldt-Jacob disease viruses and chronic infectious neuropathic agents.

[0228] Many examples of antiviral compounds that can be used in combination with the complexes of the invention are known in the art and include but are not limited to: rifampicin, nucleoside reverse transcriptase inhibitors (e.g., AZT, ddI, ddC, 3TC, d4T), non-nucleoside reverse transcriptase inhibitors (e.g., Efavirenz, Nevirapine), protease inhibitors (e.g., aprenavir, indinavir, ritonavir, and saquinavir), idoxuridine, cidofovir, acyclovir, ganciclovir, zanamivir, amantadine, and Palivizumab. Other examples of anti-viral agents include but are not limited to Acemannan; Acyclovir; Acyclovir Sodium; Adefovir; Alovudine; Alvircept Sudotox; Amantadine Hydrochloride; Aranotin; Arildone; Atevirdine Mesylate; Avridine; Cidofovir; Cipamfylline; Cytarabine Hydrochloride; Delavirdine Mesylate; Desciclovir; Didanosine; Disoxaril; Edoxudine; Enviradene; Enviroxime; Famciclovir; Famotine Hydrochloride; Fiacitabine; Fialuridine; Fosarilate; Foscamet Sodium; Fosfonet Sodium; Ganciclovir; Ganciclovir Sodium; Idoxuridine; Kethoxal; Lamivudine; Lobucavir; Memotine Hydrochloride; Methisazone; Nevirapine; Penciclovir; Pirodavir; Ribavirin; Rimantadine Hydrochloride; Saquinavir Mesylate; Somantadine Hydrochloride; Sorivudine; Statolon; Stavudine; Tilorone Hydrochloride; Trifluridine; Valacyclovir Hydrochloride; Vidarabine; Vidarabine Phosphate; Vidarabine Sodium Phosphate; Viroxime; Zalcitabine; Zidovudine; Zinviroxime.

[0229] Bacterial infections or diseases that can be treated or prevented by the methods of the present invention are caused by bacteria including, but not limited to, bacteria that have an intracellular stage in its life cycle, such as mycobacteria (e.g., Mycobacteria tuberculosis, M. bovis, M. avium, M. leprae, or M. africanum), rickettsia, mycoplasma, chlamydia, and legionella. Other examples of bacterial infections contemplated include but are not limited to infections caused by Gram positive bacillus (e.g., Listeria, Bacillus such as Bacillus anthracis, Erysipelothrix species), Gram negative bacillus (e.g., Bartonella, Brucella, Campylobacter, Enterobacter, Escherichia, Francisella, Hemophilus, Klebsiella, Morganella, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Shigella, Vibrio, and Yersinia species), spirochete bacteria (e.g., Borrelia species including Borrelia burgdorferi that causes Lyme disease), anaerobic bacteria (e.g.,

Actinomyces and Clostridium species), Gram positive and negative coccal bacteria, Enterococcus species, Streptococcus species, Pneumococcus species, Staphylococcus species, Neisseria species. Specific examples of infectious bacteria include but are not limited to: Helicobacter pyloris, Borelia burgdorferi, Legionella pneumophilia, Mycobacteria tuberculosis, M. avium, M. intracellulare, M. kansaii, M. gordonae, Staphylococcus aureus, Neisseria gonorrhoeae, Neisseria meningitidis, Listeria monocytogenes, Streptococcus pyogenes (Group A Streptococcus), Streptococcus agalactiae (Group B Streptococcus), Streptococcus viridans, Streptococcus faecalis, Streptococcus bovis, Streptococcus pneumoniae, Haemophilus influenzae, Bacillus antracis, corynebacterium diphtheriae, Erysipelothrix rhusiopathiae, Clostridium perfringers, Clostridium tetani, Enterobacter aerogenes, Klebsiella pneumoniae, Pasturella multocida, Fusobacterium nucleatum, Streptobacillus moniliformis, Treponema pallidium, Treponema pertenue, Leptospira, Rickettsia, and Actinomyces israelli.

[0230] Antibacterial agents or antibiotics that can be used in combination with the complexes of the invention include but are not limited to: aminoglycoside antibiotics (e.g., apramycin, arbekacin, bambermycins, butirosin, dibekacin, neomycin, neomycin, undecylenate, netilmicin, paromomycin, ribostamycin, sisomicin, and spectinomycin), amphenicol antibiotics (e.g., azidamfenicol, chloramphenicol, florfenicol, and thiamphenicol), ansamycin antibiotics (e.g., rifamide and rifampin), carbacephems (e.g., loracarbef), carbapenems (e.g., biapenem and imipenem), cephalosporins (e.g., cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefozopran, cefpimizole, cefpiramide, and cefpirome), cephamycins (e.g., cefbuperazone, cefmetazole, and cefminox), monobactams (e.g., aztreonam, carumonam, and tigemonam), oxacephems (e.g., flomoxef, and moxalactam), penicillins (e.g., amdinocillin, amdinocillin pivoxil, amoxicillin, bacampicillin, benzylpenicillinic acid, benzylpenicillin sodium, epicillin, fenbenicillin, floxacillin, penamccillin, penethamate hydriodide, penicillin o-benethamine, penicillin 0, penicillin V, penicillin V benzathine, penicillin V hydrabamine, penimepicycline, and phencihicillin potassium), lincosamides (e.g., clindamycin, and lincomycin), macrolides (e.g., azithromycin, carbomycin, clarithomycin, dirithromycin, erythromycin, and erythromycin acistrate), amphomycin, bacitracin, capreomycin, colistin, enduracidin, enviomycin, tetracyclines (e.g., apicycline, chlortetracycline, clomocycline, and demeclocycline), 2,4-diaminopyrimidines (e.g., brodimoprim), nitrofurans (e.g., furaltadone, and furazolium chloride), quinolones and analogs thereof (e.g., cinoxacin, ciprofloxacin, clinafloxacin, flumequine, and grepagloxacin), sulfonamides (e.g., acetyl sulfamethoxypyrazine, benzylsulfamide, noprylsulfamide, phthalylsulfacetamide,

sulfachrysoidine, and sulfacytine), sulfones (e.g., diathymosulfone, glucosulfone sodium, and solasulfone), cycloserine, mupirocin and tuberin.

[0231] Additional examples of antibacterial agents include but are not limited to Acedapsone; Acetosulfone Sodium; Alamecin; Alexidine; Amdinocillin; Amdinocillin Pivoxil; Amicycline; Amifloxacin; Amifloxacin Mesylate; Amikacin; Amikacin Sulfate; Aminosalicylic acid; Aminosalicylate sodium; Amoxicillin; Amphomycin; Ampicillin; Ampicillin Sodium; Apalcillin Sodium; Apramycin; Aspartocin; Astromicin Sulfate; Avilamycin; Avoparcin; Azithromycin; Azlocillin; Azlocillin Sodium; Bacampicillin Hydrochloride; Bacitracin; Bacitracin Methylene Disalicylate; Bacitracin Zinc; Bambermycins; Benzoylpas Calcium; Berythromycin; Betamicin Sulfate; Biapenem; Biniramycin; Biphenamine Hydrochloride; Bispyrithione Magsulfex; Butikacin; Butirosin Sulfate; Capreomycin Sulfate; Carbadox; Carbenicillin Disodium; Carbenicillin Indanyl Sodium; Carbenicillin Phenyl Sodium; Carbenicillin Potassium; Carumonam Sodium; Cefaclor; Cefadroxil; Cefamandole; Cefamandole Nafate; Cefamandole Sodium; Cefaparole; Cefatrizine; Cefazaflur Sodium; Cefazolin; Cefazolin Sodium; Cefbuperazone; Cefdinir; Cefepime; Cefepime Hydrochloride; Cefetecol; Cefixime; Cefmnenoxime Hydrochloride; Cefmetazole; Cefmetazole Sodium; Cefonicid Monosodium; Cefonicid Sodium; Cefoperazone Sodium; Ceforanide; Cefotaxime Sodium; Cefotetan; Cefotetan Disodium; Cefotiam Hydrochloride; Cefoxitin; Cefoxitin Sodium; Cefpimizole; Cefpimizole Sodium; Cefpiramide; Cefpiramide Sodium; Cefpirome Sulfate; Cefpodoxime Proxetil; Cefprozil; Cefroxadine; Cefsulodin Sodium; Ceftazidime; Ceftibuten; Ceftizoxime Sodium; Ceftriaxone Sodium; Cefuroxime; Cefuroxime Axetil; Cefuroxime Pivoxetil; Cefuroxime Sodium; Cephacetrile Sodium; Cephalexin; Cephalexin Hydrochloride; Cephaloglycin; Cephaloridine; Cephalothin Sodium; Cephapirin Sodium; Cephradine; Cetocycline Hydrochloride; Cetophenicol; Chloramphenicol; Chloramphenicol Palmitate; Chloramphenicol Pantothenate Complex; Chloramphenicol Sodium Succinate; Chlorhexidine Phosphanilate; Chloroxylenol; Chlortetracycline Bisulfate; Chlortetracycline Hydrochloride; Cinoxacin; Ciprofloxacin; Ciprofloxacin Hydrochloride; Cirolemycin; Clarithromycin; Clinafloxacin Hydrochloride; Clindamycin; Clindamycin Hydrochloride; Clindamycin Palmitate Hydrochloride; Clindamycin Phosphate; Clofazimine; Cloxacillin Benzathine; Cloxacillin Sodium; Cloxyquin; Colistimethate Sodium; Colistin Sulfate; Coumermycin; Coumermycin Sodium; Cyclacillin; Cycloserine; Dalfopristin; Dapsone; Daptomycin; Demeclocycline; Demeclocycline Hydrochloride; Demecycline; Denofungin; Diaveridine; Dicloxacillin; Dicloxacillin Sodium; Dihydrostreptomycin Sulfate; Dipyrithione; Dirithromycin; Doxycycline; Doxycycline Calcium; Doxycycline Fosfatex;

Doxycycline Hyclate; Droxacin Sodium; Enoxacin; Epicillin; Epitetracycline Hydrochloride; Erythromycin; Erythromycin Acistrate; Erythromycin Estolate; Erythromycin Ethylsuccinate; Erythromycin Gluceptate; Erythromycin Lactobionate; Erythromycin Propionate; Erythromycin Stearate; Ethambutol Hydrochloride; Ethionamide; Fleroxacin; Floxacillin; Fludalanine; Flumequine; Fosfomycin; Fosfomycin Tromethamine; Fumoxicillin; Furazolium Chloride; Furazolium Tartrate; Fusidate Sodium; Fusidic Acid; Gentamicin Sulfate; Gloximonam; Gramicidin; Haloprogin; Hetacillin; Hetacillin Potassium; Hexedine; Ibafloxacin; Imipenem; Isoconazole; Isepamicin; Isoniazid; Josamycin; Kanamycin Sulfate; Kitasamycin; Levofuraltadone; Levopropylcillin Potassium; Lexithromycin; Lincomycin; Lincomycin Hydrochloride; Lomefloxacin; Lomefloxacin Hydrochloride; Lomefloxacin Mesylate; Loracarbef; Mafenide; Meclocycline; Meclocycline Sulfosalicylate; Megalomicin Potassium Phosphate; Mequidox; Meropenem; Methacycline; Methacycline Hydrochloride; Methenamine; Methenamine Hippurate; Methenamine Mandelate; Methicillin Sodium; Metioprim; Metronidazole Hydrochloride; Metronidazole Phosphate; Mezlocillin; Mezlocillin Sodium; Minocycline; Minocycline Hydrochloride; Mirincamycin Hydrochloride; Monensin; Monensin Sodium; Nafcillin Sodium; Nalidixate Sodium; Nalidixic Acid; Natamycin; Nebramycin; Neomycin Palmitate; Neomycin Sulfate; Neomycin Undecylenate; Netilmicin Sulfate; Neutramycin; Nifuradene; Nifuraldezone; Nifuratel; Nifuratrone; Nifurdazil; Nifurimide; Nifurpirinol; Nifurquinazol; Nifurthiazole; Nitrocycline; Nitrofurantoin; Nitromide; Norfloxacin; Novobiocin Sodium; Ofloxacin; Ormetoprim; Oxacillin Sodium; Oximonam; Oximonam Sodium; Oxolinic Acid; Oxytetracycline; Oxytetracycline Calcium; Oxytetracycline Hydrochloride; Paldimycin; Parachlorophenol; Paulomycin; Pefloxacin; Pefloxacin Mesylate; Penamecillin; Penicillin G Benzathine; Penicillin G Potassium; Penicillin G Procaine; Penicillin G Sodium; Penicillin V; Penicillin V Benzathine; Penicillin V Hydrabamine; Penicillin V Potassium; Pentizidone Sodium; Phenyl Aminosalicylate; Piperacillin Sodium; Pirbenicillin Sodium; Piridicillin Sodium; Pirlimycin Hydrochloride; Pivampicillin Hydrochloride; Pivampicillin Pamoate; Pivampicillin Probenate; Polymyxin B Sulfate; Porfiromycin; Propikacin; Pyrazinamide; Pyrithione Zinc; Quindecamine Acetate; Quinupristin; Racephenicol; Ramoplanin; Ranimycin; Relomycin; Repromicin; Rifabutin; Rifametane; Rifamexil; Rifamide; Rifampin; Rifapentine; Rifaximin; Rolitetracycline; Rolitetracycline Nitrate; Rosaramicin; Rosaramicin Butyrate; Rosaramicin Propionate; Rosaramicin Sodium Phosphate; Rosaramicin Stearate; Rosoxacin; Roxarsone; Roxithromycin; Sancycline; Sanfetrinem Sodium; Sarmoxicillin; Sarpicillin; Scopafingin; Sisomicin; Sisomicin Sulfate; Sparfloxacin; Spectinomycin

Hydrochloride; Spiramycin; Stallimycin Hydrochloride; Steffimycin; Streptomycin Sulfate; Streptonicozid; Sulfabenz; Sulfabenzamide; Sulfacetamide; Sulfacetamide Sodium; Sulfacytine; Sulfadiazine; Sulfadiazine Sodium; Sulfadoxine; Sulfalene; Sulfamerazine; Sulfameter; Sulfamethazine; Sulfamethizole; Sulfamethoxazole; Sulfamonomethoxine; Sulfamoxole; Sulfanilate Zinc; Sulfanitran; Sulfasalazine; Sulfasomizole; Sulfathiazole; Sulfazamet; Sulfisoxazole; Sulfisoxazole Acetyl; Sulfisoxazole Diolamine; Sulfomyxin; Sulopenem; Sultamicillin; Suncillin Sodium; Talampicillin Hydrochloride; Teicoplanin; Temafloxacin Hydrochloride; Temocillin; Tetracycline; Tetracycline Hydrochloride; Tetracycline Phosphate Complex; Tetroxoprim; Thiamphenicol; Thiphencillin Potassium; Ticarcillin Cresyl Sodium; Ticarcillin Disodium; Ticarcillin Monosodium; Ticlatone; Tiodonium Chloride; Tobramycin; Tobramycin Sulfate; Tosufloxacin; Trimethoprim; Trimethoprim Sulfate; Trisulfapyrimidines; Troleandomycin; Trospectomycin Sulfate; Tyrothricin; Vancomycin; Vancomycin Hydrochloride; Virginiamycin; Zorbamycin.

[0232] Fungal diseases that can be treated or prevented by the methods of the present invention include but not limited to aspergilliosis, crytococcosis, sporotrichosis, coccidioidomycosis, paracoccidioidomycosis, histoplasmosis, blastomycosis, zygomycosis, and candidiasis.

[0233] Antifungal compounds that can be used in combination with the complexes of the invention include but are not limited to: polyenes (e.g., amphotericin b, candicidin, mepartricin, natamycin, and nystatin), allylamines (e.g., butenafine, and naftifine), imidazoles (e.g., bifonazole, butoconazole, chlordantoin, flutrimazole, isoconazole, ketoconazole, and lanoconazole), thiocarbamates (e.g., tolciclate, tolindate, and tolnaftate), triazoles (e.g., fluconazole, itraconazole, saperconazole, and terconazole), bromosalicylchloranilide, buclosamide, calcium propionate, chlorphenesin, ciclopirox, azaserine, griseofulvin, oligomycins, neomycin undecylenate, pyrrolnitrin, siccanin, tubercidin, and viridin. Additional examples of antifungal compounds include but are not limited to Acrisorcin; Ambruticin; Amphotericin B; Azaconazole; Azaserine; Basifungin; Bifonazole; Biphenamine Hydrochloride; Bispyrithione Magsulfex; Butoconazole Nitrate; Calcium Undecylenate; Candicidin; Carbol-Fuchsin; Chlordantoin; Ciclopirox; Ciclopirox Olamine; Cilofungin; Cisconazole; Clotrimazole; Cuprimyxin; Denofungin; Dipyrithione; Doconazole; Econazole; Econazole Nitrate; Enilconazole; Ethonam Nitrate; Fenticonazole Nitrate; Filipin; Fluconazole; Flucytosine; Fungimycin; Griseofulvin; Hamycin; Isoconazole; Itraconazole; Kalafungin; Ketoconazole; Lomofingin; Lydimycin; Mepartricin; Miconazole; Miconazole Nitrate; Monensin; Monensin Sodium; Naftifine Hydrochloride; Neomycin Undecylenate; Nifuratel; Nifurmerone; Nitralamine

Hydrochloride; Nystatin; Octanoic Acid; Orconazole Nitrate; Oxiconazole Nitrate; Oxifungin Hydrochloride; Parconazole Hydrochloride; Partricin; Potassium Iodide; Proclonol; Pyrithione Zinc; Pyrrolnitrin; Rutamycin; Sanguinarium Chloride; Saperconazole; Scopafungin; Selenium Sulfide; Sinefungin; Sulconazole Nitrate; Terbinafine; Terconazole; Thiram; Ticlatone; Tioconazole; Tolciclate; Tolindate; Tolnaftate; Triacetin; Triafuigin; Undecylenic Acid; Viridoflilvin; Zinc Undecylenate; and Zinoconazole Hydrochloride.

[0234] Parasitic diseases that can be treated or prevented by the methods of the present invention including, but not limited to, amebiasis, malaria, leishmania, coccidia, giardiasis, cryptosporidiosis, toxoplasmosis, and trypanosomiasis. Also encompassed are infections by various worms, such as but not limited to ascariasis, ancylostomiasis, trichuriasis, strongyloidiasis, toxoccariasis, trichinosis, onchocerciasis. filaria, and dirofilariasis. Also encompassed are infections by various flukes, such as but not limited to schistosomiasis, paragonimiasis, and clonorchiasis.

[0235] Many examples of antiprotozoal compounds that can be used in combination with the complexes of the invention to treat parasitic diseases are known in the art and include but are not limited to: quinines, chloroquine, mefloquine, proguanil, pyrimethamine, metronidazole, diloxanide furoate, tinidazole, amphotericin, sodium stibogluconate, trimoxazole, and pentamidine isetionate. Many examples of antiparasite drugs that can be used in combination with the complexes of the invention to treat parasitic diseases are known in the art and include but are not limited to: mebendazole, levamisole, niclosamide, praziquantel, albendazole, ivermectin, diethylcarbamazine, and thiabendazole. Further examples of anti-parasitic compounds include but are not limited to Acedapsone; Amodiaquine Hydrochloride; Amquinate; Arteflene; Chloroquine; Chloroquine Hydrochloride; Chloroquine Phosphate; Cycloguanil Pamoate; Enpiroline Phosphate; Halofantrine Hydrochloride; Hydroxychloroquine Sulfate; Mefloquine Hydrochloride; Menoctone; Mirincamycin Hydrochloride; Primaquine Phosphate; Pyrimethamine; Quinine Sulfate; and Tebuquine.

[0236] In a preferred embodiment, the complexes of the invention are administered in combination with one or more antibacterial, antiviral, antifungal, or antiprotozoal agents.

[0237] In a certain embodiment, the complexes of the invention can be used in combination with a non-HSP and non-α2M-based vaccine composition. Examples of such vaccines for humans are described in The Jordan Report 2000, Accelerated Development of Vaccines, National Institute of Health, which is incorporated herein by reference in its entirety. Many vaccines for the treatment of non-human vertebrates are disclosed in

Bennett, K. Compendium of Veterinary Products, 3rd ed. North American Compendiums, Inc., 1995, which is incorporated herein by reference in its entirety.

[0238] Veterinary diseases that can be treated or prevented by the methods of the present invention include but are not limited to diseases caused by parasites. Typical parasites infecting swine include Eimeria bebliecki, Eimeria scabra, Isospora suis, Giardia spp.; Balantidium coli, Entamoeba histolytica; Toxoplasma gondii and Sarcocystis spp., and Trichinella spiralis. The major parasites of dairy and beef cattle include Eimeria spp., Cryptosporidium spp., Giardia spp., Toxoplasma gondii; Babesia bovis (RBC), Babesia bigemina (RBC), Trypanosoma spp. (plasma), Theileria spp. (RBC); Theileria parva (lymphocytes); Tritrichomonas foetus; and Sarcocystis spp.

[0239] The major parasites of raptors include Trichomonas gallinae; Coccidia (Eimeria spp.); Plasmodium relictum, Leucocytozoon danilewskyi (owls), Haemoproteus spp., Trypanosoma spp.; Histomonas; Cryptosporidium meleagridis, Cryptosporidium baileyi, Giardia, Eimeria; Toxoplasma. Typical parasites infecting sheep and goats include Eimeria spp., Cryptosporidium spp., Giardia spp.; Toxoplasma gondii; Babesia spp. (RBC), Trypanosoma spp. (plasma), Theileria spp. (RBC); and Sarcocystis spp.

[0240] Typical parasitic infections in poultry include coccidiosis caused by Eimeria acervulina, E. necatrix, E. tenella, Isospora spp. and Eimeria truncata; histomoniasis, caused by Histomonas meleagridis and Histomonas gallinarum; trichomoniasis caused by Trichomonas gallinae; and hexamitiasis caused by Hexamita meleagridis. Poultry can also be infected Emeria maxima, Emeria meleagridis, Eimeria adenoeides, Eimeria meleagrimitis, Cryptosporidium, Eimeria brunetti, Emeria adenoeides, Leucocytozoon spp., Plasmodium spp., Hemoproteus meleagridis, Toxoplasma gondii and Sarcocystis.

[0241] Parasitic infections also pose serious problems in laboratory research settings involving animal colonies. Some examples of laboratory animals intended to be treated, or in which parasite infection is sought to be prevented, by the methods of the invention include mice, rats, rabbits, guinea pigs, nonhuman primates, as well as the aforementioned swine and sheep. Typical parasites in mice include Leishmania spp., Plasmodium berghei, Plasmodium yoelii, Giardia muris, Hexamita muris; Toxoplasma gondii; Trypanosoma duttoni (plasma); Kiossiella muris; Sarcocystis spp. Typical parasites in rats include Giardia muris, Hexamita muris; Toxoplasma gondii; Trypanosoma lewisi (plasma); Trichinella spiralis; Sarcocystis spp. Typical parasites in rabbits include Eimeria spp.; Toxoplasma gondii; Nosema cuniculi; Eimeria stiedae, Sarcocystis spp. Typical parasites of the hamster include Trichomonas spp.; Toxoplasma gondii; Trichinella spiralis; Sarcocystis spp. Typical

parasites in the guinea pig include Balantidium caviae; Toxoplasma gondii; Klossiella caviae; Sarcocystis spp.

[0242] The methods of the invention can also be applied to the treatment and/or prevention of parasitic infection in dogs, cats, birds, fish and ferrets. Typical parasites of birds include Trichomonas gallinae; Eimeria spp., Isospora spp., Giardia; Cryptosporidium; Sarcocystis spp., Toxoplasma gondii, Haemoproteus/Parahaemoproteus, Plasmodium spp., LeucocytozoonlAkiba, Atoxoplasma, Trypanosoma spp. Typical parasites infecting dogs include Trichinella spiralis; Isopora spp., Sarcocystis spp., Cryptosporidium spp., Hammondia spp., Giardia duodenalis (canis); Balantidium coli, Entamoeba histolytica; Hepatozoon canis; Toxoplasma gondii, Trypanosoma cruzi; Babesia canis, Leishmania amastigotes; Neospora caninum.

[0243] Typical parasites infecting feline species include Isospora spp., Toxoplasma gondii, Sarcocystis spp., Hammondia hammondi, Besnoitia spp., Giardia spp.; Entamoeba histolytica; Hepatozoon canis, Cytauxzoon spp., Cytauxzoon spp., Cytauxzoon spp. (red cells, RE cells).

[0244] Typical parasites infecting fish include Hexamita spp., Eimeria spp.; Cryptobia spp., Nosema spp., Myxosoma spp., Chilodonella spp., Trichodina spp.; Plistophora spp., Myxosoma Henneguya; Costia spp., Ichthyophithirius spp., and Oodinium spp.

[0245] Typical parasites of wild mammals include Giardia spp. (carnivores, herbivores), Isospora spp. (carnivores), Eimeria spp. (carnivores, herbivores); Theileria spp. (herbivores), Babesia spp. (carnivores, herbivores), Trypanosoma spp. (carnivores, herbivores); Schistosoma spp. (herbivores); Fasciola hepatica (herbivores), Fascioloides magna (herbivores), Fasciola gigantica (herbivores), Trichinella spiralis (carnivores, herbivores).

[0246] Parasitic infections in zoos can also be treated by the methods of the invention. Typical parasites of the Bovidae family (blesbok, antelope, banteng, eland, gaur, impala, klipspringer, kudu, gazelle) include Eimeria spp. Typical parasites in the Pinnipedae family (seal, sea lion) include Eimeria phocae. Typical parasites in the Camelidae family (camels, llamas) include Eimeria spp. Typical parasites of the Giraffidae family (giraffes) include Eimeria spp. Typical parasites in the Elephantidae family (African and Asian) include Fasciola spp. Typical parasites of lower primates (chimpanzees, orangutans, apes, baboons, macaques, monkeys) include Giardia spp.; Balantidium coli, Entamzoeba histolytica, Sarcocystis spp., Toxoplasma gondii; Plasmodim spp. (RBC), Babesia spp. (RBC), Trypanosoma spp. (plasma), Leishmania spp. (macrophages).

5.6. ADOPTIVE IMMUNOTHERAPY

[0247] Adoptive immunotherapy refers to a therapeutic approach for treating cancer or infectious diseases in which immune cells are administered to a host with the aim that the cells mediate either directly or indirectly specific immunity to tumor cells and/or antigenic components or regression of the tumor or treatment of infectious diseases, as the case may be. (See e.g., U.S. Patent No. 5,985,270, issued November 16, 1999, which is incorporated by reference herein in its entirety).

[0248] In one embodiment, antigen presenting cells (APC) for use in adoptive immunotherapy are sensitized with HSPs and/or α2M complexed with one or more antigenic sets of peptides prepared in accordance with the methods described herein. The complexes can be produced by complexing heat shock protein or alpha-2-macroglobulin to an antigenic set of peptides. The complexes are produced by (a) subjecting a target antigen derived from cells of said type of cancer to either digestion with a protease to generate a antigenic set of peptides, and (b) complexing the antigenic set of peptides to heat shock protein or alpha-2-macroglobulin.

[0249] In another embodiment, therapy by administration of in vitro complexed antigenic set of peptides and HSPs and/or α2M prepared by the methods of the invention may be combined with adoptive immunotherapy using APC sensitized by HSP- and/or α2M-peptide complexes prepared by any method known in the art (see e.g., U.S. Patent No. 5,985,270) in which the antigenic set of peptides display the desired antigenicity (e.g., of the type of cancer or pathogen). The sensitized APC can be administered alone, in combination with the in vitro complexed antigenic set of peptides and HSPs and/or α2M, or before or after administration of the complexed antigenic set of peptides and HSPs and/or α2M. In particular, the use of sensitized APC to prevent and treat cancer can further comprise administering to the subject an amount, effective for said treatment or prevention, of complexes comprising heat shock protein and/or alpha-2-macroglobulin, complexed to antigenic sets of peptides, wherein said complexes were produced as described above. Similarly, the use of sensitized APC in treating or preventing a type of infectious disease, can further comprise administering to the subject an amount, effective for said treatment or prevention, of complexes comprising heat shock protein and/or alpha-2-macroglobulin, complexed to antigenic sets of peptides.

[0250] Furthermore, the mode of administration of the in vitro complexed antigenic sets of peptides and HSPs and/or α2M can be varied, including but not limited to, e.g., subcutaneously, intravenously or intramuscularly, although intradermally is preferred. In another specific embodiment, adoptive immunotherapy by administration of the antigen

presenting cells sensitized with complexes made according to the present invention can be combined with therapy by administration by HSP- and/or α2M-peptide complexes prepared by any method known in the art (see e.g., U.S. Patent No. 5,750,119, 5,837,251, 5,961,979, 5,935,576, PCT publications WO 94/14976 or WO 99/50303) in which the antigenic molecules display the desired antigenicity (e.g., of the type of cancer or pathogen).

5.6.1. OBTAINING ANTIGEN-PRESENTING CELLS

[0251] The antigen-presenting cells, including but not limited to macrophages, dendritic cells and B-cells, are preferably obtained by production in vitro from stem and progenitor cells from human peripheral blood or bone marrow as described by Inaba, K., et al., 1992, J. Exp. Med. 176:1693-1702. Dendritic cells can be obtained by any of various methods known in the art. By way of example but not limitation, dendritic cells can be obtained by the methods described in Sallusto et al., 1994, J Exp Med 179:1109-1118 and Caux et al., 1992, Nature 360, 258-261 which are incorporated herein by reference in their entireties. In a preferred aspect, human dendritic cells obtained from human blood cells are used.

[0252] APC can be obtained by any of various methods known in the art. In one aspect, human macrophages are used, obtained from human blood cells. By way of example but not limitation, macrophages can be obtained as follows:

[0253] Mononuclear cells are isolated from peripheral blood of a patient (preferably the patient to be treated), by Ficoll-Hypaque gradient centrifugation and are seeded on tissue culture dishes which are pre-coated with the patient's own serum or with other AB+ human serum. The cells are incubated at 37°C for 1 hour, then non-adherent cells are removed by pipetting. To the adherent cells left in the dish, is added cold (4°C) 1 mM EDTA in phosphate-buffered saline and the dishes are left at room temperature for 15 minutes. The cells are harvested, washed with RPMI buffer and suspended in RPMI buffer. Increased numbers of macrophages may be obtained by incubating at 37°C with macrophage-colony stimulating factor (M-CSF).

5.6.2. SENSITIZATION OF MACROPHAGES AND ANTIGEN PRESENTING CELLS WITH HSP-PEPTIDE OR α2M-PEPTIDE COMPLEXES

[0254] APC are sensitized with HSP or α2M bound to antigenic sets of peptides preferably by incubating the cells in vitro with the complexes. The APC are sensitized with complexes of HSPs or α2M and antigenic sets of peptides by incubating in vitro with the HSP-complex or α2M-complex at 37°C for 15 minutes to 24 hours. By way of example but not limitation, $4x10^7$ dendritic cells can be incubated with 10 microgram gp96-peptide

complexes per ml or 100 microgram HSP90-peptide complexes per ml at 37°C for 15 minutes-24 hours in 1 ml plain RPMI medium. The cells are washed three times and resuspended in a physiological medium preferably sterile, at a convenient concentration (e.g., $1x10^7$/ml) for injection in a patient. Preferably, the patient into which the sensitized dendritic cells are injected is the patient from which the dendritic cells were originally isolated (autologous embodiment).

[0255] Optionally, the ability of sensitized APC to stimulate, for example, the antigen-specific, class I-restricted cytotoxic T-lymphocytes (CTL) can be monitored by their ability to stimulate CTLs to release tumor necrosis factor, and by their ability to act as targets of such CTLs.

5.6.3. REINFUSION OF SENSITIZED APC

[0256] The sensitized APCs are reinfused into the patient systemically, preferably intradermally, by conventional clinical procedures. These activated cells are reinfused, preferentially by systemic administration into the autologous patient. Patients generally receive from about 10^6 to about 10^{12} sensitized dendritic cells depending on the condition of the patient. In some regimens, patients may optionally receive in addition a suitable dosage of a biological response modifier including but not limited to the cytokines IFN-α, IFN-γ, IL-2, IL-4, IL-6, TNF or other cytokine growth factor.

5.7. PHARMACEUTICAL PREPARATIONS AND METHODS OF ADMINISTRATION

[0257] The invention provides complexes of antigenic sets of peptides bound to HSPs and/or α2M prepared by the methods of the invention, and pharmaceutical compositions comprising the complexes. In certain embodiments, the complexes in the pharmaceutical compositions are purified. The complexes and pharmaceutical compositions of the invention can be administered to a patient at therapeutically effective doses to treat or ameliorate a cell proliferative disorder or infectious disease. A therapeutically effective dose refers to that amount of the complexes sufficient to result in amelioration of symptoms of such a disorder. The effective dose of the complexes may be different when another treatment modality is being used in combination. The appropriate and recommended dosages, formulation and routes of administration for treatment modalities such as chemotherapeutic agents, radiation therapy and biological/immunotherapeutic agents such as cytokines are known in the art and described in such literature as the Physician's Desk Reference (56^{th} ed., 2002).

5.7.1. EFFECTIVE DOSE

[0258] The compositions of the present invention, comprising an immunogenic, effective amount of complexes of a population of antigenic peptides with HSP and/or α2M are administered to a subject in need of treatment against cancer or an infectious disease, as a method of inducing an immune response against that cancer or infectious disease. Toxicity and therapeutic efficacy of such complexes can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Complexes that exhibit large therapeutic indices are preferred. While complexes that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such complexes to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[0259] In one embodiment, the data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of complexes lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any complexes used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[0260] In another embodiment, an amount of hsp70- and/or gp96-antigenic molecule complexes is administered that is in the range of about 0.1 microgram to about 600 micrograms, and preferably about 1 micrograms to about 60 micrograms for a human patient. The amount of hsp70- and/or gp96 complexes administered is 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 250, 300, 400, 500 or 600 micrograms. Preferably, the amount is less than 100 micrograms. Most preferably, the amount of hsp70- and/or gp96 complexes administered is 5 micrograms, 25 micrograms, or 50 micrograms. The dosage for hsp-90 peptide complexes in a human patient provided by the present invention is in the range of about 5 to 5,000 micrograms. Preferably, the the amount of hsp90 complexes administered is 5, 10, 25, 50, 60, 70, 80, 90, 100, 200, 250, 500, 1000,

2000, 2500, or 3000 microgram, the most preferred dosage being 100 microgram. In preferred aspects, an amount of a composition comprising α2M complex is administered to a human that is in the range of about 1 microgram to 5 milligram, preferably 10 to 200 microgram, preferably 10, 20, 25, 50, 100, or 200 microgram.

[0261] These doses are preferably administered intradermally or subcutaneously. These doses can be given once or repeatedly, such as daily, every other day, weekly, biweekly, or monthly. Preferably, the complexes are given once weekly for a period of about 4-6 weeks, and the mode or site of administration is preferably varied with each administration. Thus, by way of example and not limitation, the first injection may be given subcutaneously on the left arm, the second on the right arm, the third on the left belly, the fourth on the right belly, the fifth on the left thigh, the sixth on the right thigh, etc. The same site may be repeated after a gap of one or more injections. Also, split injections may be given. Thus, for example, half the dose may be given in one site and the other half on an other site on the same day. Alternatively, the mode of administration is sequentially varied, e.g., weekly injections are given in sequence intradermally, intramuscularly, subcutaneously, intravenously or intraperitoneally. Preferably, the once weekly dose is given for a period of 4 weeks. After 4-6 weeks, further injections are preferably given at two-week intervals over a period of time of one or more months, or until supply of complexes is exhausted. Later injections may be given monthly. The pace of later injections may be modified, depending upon the patient's clinical progress and responsiveness to the immunotherapy. In a preferred example, intradermal administrations are given, with each site of administration varied sequentially.

[0262] In various embodiments, sites of vaccination may include but are not limited to the anterior deltoid regions, the subclavicular region bilaterally, and the medial inguinal regions of the upper thighs. Areas distal to lymph node basins that have been resected or irradiated or areas just distal to a surgical scar preferably should not be injected. Preferably, the sites of injections should be rotated, so that injections are not repeated at the same site twice in a row, and all potential sites are used before any sites are repeated. The injection may be given into one site or into two adjacent sites (0.2 ml each) a few centimeters apart. In a specific embodiment, a composition of the invention is not administered orally. In another specific embodiment, a composition of the invention is not administered mucosally.

[0263] Accordingly, the invention provides methods of preventing and treating cancer or an infectious disease in a subject comprising administering a composition which stimulates the immunocompetence of the host individual and elicits specific immunity against the preneoplastic and/or neoplastic cells or infected cells.

[0264] In a specific embodiment, during combination therapy, the HSP complexes or α2M complexes are administered in a sub-optimal amount, e.g., an amount that does not manifest detectable therapeutic benefits when administered in the absence of the other therapeutic modality, as determined by methods known in the art. In such methods, the administration of such a sub-optimal amount of HSP complexes to a subject receiving another therapeutic modality results in an overall improvement in effectiveness of treatment. In another specific embodiment, the α2M complexes are administered in a sub-optimal amount during combination therapy. In such methods, the administration of such a sub-optimal amount of α2M complexes to a subject receiving a therapeutic modality results in an overall improvement in effectiveness of treatment.

[0265] In a preferred embodiment, an HSP complexes are administered in an amount that does not result in tumor regression or cancer remission or an amount wherein the cancer cells have not been significantly reduced or have increased when said HSP complexes are administered in the absence of the therapeutic modality. In a preferred embodiment, the sub-optimal amount of HSP complexes are administered to a subject receiving a treatment modality whereby the overall effectiveness of treatment is improved. In another preferred embodiment, an α2M complexes are administered in an amount that does not result in tumor regression or cancer remission or an amount wherein the cancer cells have not been significantly reduced or have increased when said α2M complexes are administered in the absence of the therapeutic modality. In a preferred embodiment, the sub-optimal amount of α2M complexes are administered to a subject receiving a treatment modality whereby the overall effectiveness of treatment is improved. Among these subjects being treated with HSP or α2M complexes are those receiving chemotherapy or radiation therapy. A sub-optimal amount can be determined by appropriate animal studies. Such a sub-optimal amount in humans can be determined by extrapolation from experiments in animals.

[0266] In certain specific embodiments, an HSP or α2M complexes are administered to a subject already receiving a chemotherapeutic agent, such as Gleevec™ (e.g., 400-800 mg daily in capsule form, 400-600 mg doses administered once daily, or 800 mg dose administered daily in two doses of 400 mg each). Gleevec™ is used hereinbelow as a non-limiting example of a chemotherapeutic agent that can be used in combination. For many other chemotherapeutic agents, a similar dosing regime can be used. In such embodiments, the appropriate HSP/α2M complexes are initially administered to a subject who has already been receiving Gleevec™ in the absence of HSP/α2M complexes 2 days, 2 days to 1 week, 1 week to 1 month, 1 month to 6 months, 6 months to 1 year prior to administration of

HSP/α2M complexes in addition to Gleevec™. In a specific embodiment, HSP/α2M complexes are administered to a subject wherein the subject showed resistance to treatment with Gleevec™ alone.

[0267] In other embodiments, HSP/α2M complexes are initially administered to a subject concurrently with the initial administration of Gleevec™.

[0268] In yet other specific embodiments, Gleevec™ (e.g., 400-800 mg daily in capsule form) is administered to a subject already receiving treatment comprising administration of HSP/α2M complexes. In such embodiments, Gleevec™ is initially administered to a subject who has already been receiving HSP/α2M complexes in the absence of Gleevec™ 2 days, 2 days to 1 week, 1 week to 1 month, 1 month to 6 months, 6 months to 1 year prior to administration of Gleevec™ in addition to administration of HSP/α2M complexes.

[0269] In a specific embodiment, a chemotherapeutic agent such as Gleevec™ is administered orally. In another specific embodiment, the HSP/α2M complexes are administered intradermally.

[0270] In each of the methods contemplated above, the subject, by way of example, receives 50 mg to 100 mg, 100 mg to 200 mg, 200 mg to 300 mg, 300 mg to 400 mg, 400 mg to 500 mg, 500 mg to 600 mg, 600 mg to 700 mg, 700 mg to 800 mg, 800 mg to 900 mg, or 900 mg to 1000 mg of chemotherapeutic agents, such as Gleevec™ , daily. In certain embodiments, the total daily dose is administered to a subject as two daily doses of 25mg to 50 mg, 50 mg to 100 mg, 100 mg to 200 mg, 200 mg to 300 mg, 300 mg to 400 mg, or 400 mg to 500 mg.

5.7.2. THERAPEUTIC REGIMENS

[0271] For any of the combination therapies described above for treatment or prevention of cancer and infectious diseases, the complexes of the invention can be administered prior to, concurrently with, or subsequent to the administration of the non-HSP and non-α2M based modality. The non-HSP and non-α2M based modality can be any one of the modalities described above for treatment or prevention of cancer or infectious disease.

[0272] In one embodiment, the complexes of the invention are administered to a subject at reasonably the same time as the other modality. This method provides that the two administrations are performed within a time frame of less than one minute to about five minutes, or up to about sixty minutes from each other, for example, at the same doctor's visit.

[0273] In another embodiment, the complexes of the invention and a modality are administered at exactly the same time. In yet another embodiment the complexes of the invention and the modality are administered in a sequence and within a time interval such that the complexes of the invention and the modality can act together to provide an increased benefit than if they were administered alone. In another embodiment, the complexes of the invention and a modality are administered sufficiently close in time so as to provide the desired therapeutic or prophylactic outcome. Each can be administered simultaneously or separately, in any appropriate form and by any suitable route. In one embodiment, the complexes of the invention and the modality are administered by different routes of administration. In an alternate embodiment, each is administered by the same route of administration. The complexes of the invention can be administered at the same or different sites, e.g. arm and leg. When administered simultaneously, the complexes of the invention and the modality may or may not be administered in admixture or at the same site of administration by the same route of administration. Preferably, each is administered in the vicinity of the same draining lymph node.

[0274] In various embodiments, the complexes of the invention and the modality are administered less than 1 hour apart, at about 1 hour apart, 1 hour to 2 hours apart, 2 hours to 3 hours apart, 3 hours to 4 hours apart, 4 hours to 5 hours apart, 5 hours to 6 hours apart, 6 hours to 7 hours apart, 7 hours to 8 hours apart, 8 hours to 9 hours apart, 9 hours to 10 hours apart, 10 hours to 11 hours apart, 11 hours to 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In other embodiments, the complexes of the invention and vaccine composition are administered 2 to 4 days apart, 4 to 6 days apart, 1 week a part, 1 to 2 weeks apart, 2 to 4 weeks apart, one moth apart, 1 to 2 months apart, or 2 or more months apart. In preferred embodiments, the complexes of the invention and the modality are administered in a time frame where both are still active. One skilled in the art would be able to determine such a time frame by determining the half life of each administered component.

[0275] In one embodiment, the complexes of the invention and the modality are administered within the same patient visit. In a specific preferred embodiment, the complexes of the invention is administered prior to the administration of the modality. In an alternate specific embodiment, the complexes of the invention is administered subsequent to the administration of the modality.

[0276] In certain embodiments, the complexes of the invention and the modality are cyclically administered to a subject. Cycling therapy involves the administration of the complexes of the invention for a period of time, followed by the administration of a

modality for a period of time and repeating this sequential administration. Cycling therapy can reduce the development of resistance to one or more of the therapies, avoid or reduce the side effects of one of the therapies, and/or improve the efficacy of the treatment. In such embodiments, the invention contemplates the alternating administration of a complexes of the invention followed by the administration of a modality 4 to 6 days later, preferable 2 to 4 days, later, more preferably 1 to 2 days later, wherein such a cycle may be repeated as many times as desired. In certain embodiments, the complexes of the invention and the modality are alternately administered in a cycle of less than 3 weeks, once every two weeks, once every 10 days or once every week. In a specific embodiment, complexes of the invention is administered to a subject within a time frame of one hour to twenty four hours after the administration of a modality. The time frame can be extended further to a few days or more if a slow- or continuous-release type of modality delivery system is used.

5.7.3. FORMULATIONS AND USE

[0277] Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Preferred examples of carrier include is not limited to (i) Phosphate Buffered Saline (PBS); (ii) 10mM KPO_4, 150mM NaCl; (iii) 10mM HEPES, 150mM NaCl; (iv) 10mM imidazole, 150mM NaCl; and (v) 20mM sodium citrate. Excipients that can be used include but is not limited to (i) glycerol (10%, 20%); (ii) Tween 50 (0.05%, 0.005%); (iii) 9% sucrose; (iv) 20% sorbitol; (v) 10mM lysine; or (vi) 0.01mM dextran sulfate. In a preferred embodiment, the composition of the invention comprising heat shock protein or α2M complexes further comprises 9% sucrose, 5-10 mM potassium phosphate. In a related preferred embodiment, the pH of the composition of the invention is 7.

[0278] In various embodiments, the complexes and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) oral, buccal, parenteral, rectal, or transdermal administration. Non-invasive methods of administration are also contemplated.

[0279] For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well

known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[0280] Preparations for oral administration may be suitably formulated to give controlled release of the active complexes.

[0281] For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0282] For administration by inhalation, the complexes for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the complexes and a suitable powder base such as lactose or starch.

[0283] The complexes may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0284] The complexes may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0285] In addition to the formulations described previously, the complexes may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the complexes may be formulated with suitable polymeric or

hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0286] The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

[0287] Also encompassed is the use of adjuvants in combination with or in admixture with the complexes of the invention. Adjuvants contemplated include but are not limited to mineral salt adjuvants or mineral salt gel adjuvants, particulate adjuvants, microparticulate adjuvants, mucosal adjuvants, and immunostimulatory adjuvants. Adjuvants can be administered to a subject as a mixture with complexes of the invention, or used in combination with the complexes.

[0288] Also contemplated is the use of adenosine diphosphate (ADP) in combination with or in admixture with the complexes of the invention, preferably gp96 complexes.

5.7.4. KITS

[0289] The invention also provides kits for carrying out the methods and/or therapeutic regimens of the invention.

[0290] In one embodiment, such kits comprise in one or more containers a target antigen which is to be fragmented into an antigenic set of peptides for combining with HSPs and/or α2M that are provided in a second container. In another embodiment, such kits comprise in one or more containers antigenic sets of peptides comprising peptides of a target antigen for combining with HSPs and/or α2M that are provided in a second container. Optionally, a purified HSP for complexing to peptides is further provided in a second container. Optionally, one or more proteolytic compositions comprising one or more proteases or non-enzymatic cleavage agent are further provided in another container.

[0291] In another embodiment, such kits comprise in one or more containers therapeutically or prophylactically effective amounts of the complexed antigenic sets of peptides to HSPs and/or α2M, preferably purified, in pharmaceutically acceptable form. The kits optionally further comprise in a second container sensitized APCs, preferably purified.

[0292] The HSP or α2M complexes in a container of a kit of the invention may be in the form of a pharmaceutically acceptable solution, e.g., in combination with sterile saline, dextrose solution, or buffered solution, or other pharmaceutically acceptable sterile fluid. Alternatively, the HSP and α2M complexes may be lyophilized or desiccated; in this

instance, the kit optionally further comprises in a container a pharmaceutically acceptable solution (e.g., saline, dextrose solution, etc.), preferably sterile, to reconstitute the HSPs and α2M or α2M and HSP-containing complexes to form a solution for injection purposes.

[0293] In another embodiment, a kit of the invention further comprises a needle or syringe, preferably packaged in sterile form, for injecting the HSP and α2M complex, and/or a packaged alcohol pad. Instructions are optionally included for administration of α2M and HSP-peptide complexes by a clinician or by the patient.

[0294] Kits are also provided for carrying out the combination therapies of the present invention. In one embodiment, a kit comprises a first container containing a purified HSP complexes or α2M prepration and a second container containing a non-HSP and non-α2M based therapeutic modality for treatment of cancer. Preferably, the cancer is CML, the HSP complexes comprises hsp70-peptide complexes, and the therapeutic modality is Gleevec™. In a specific embodiment, the second container contains imatinib mesylate. In another specific embodiment, the imatinib mesylate is purified.

[0295] In a specific embodiment, a kit comprises a first container containing a purified HSP complexes or α2M complexes in an amount ineffective to treat a disease or disorder when administered alone; and a second container containing a non-HSP and non-α2M based treatment modality in an amount that, when administered before, concurrently with, or after the administration of the HSP complexes or α2M complexes in the first container, is effective to improve overall treatment effectiveness over the effectiveness of the administration of each component alone. In another specific embodiment, a kit comprises a first container containing a purified HSP complexes or α2M complexes in an amount ineffective to treat a disease or disorder when administered alone; and a second container containing one or more non-HSP and non-α2M based treatment modalities in an amount that, when administered before, concurrently with, or after the administration of the HSP complexes or α2M complexes in the first container, is effective to improve overall treatment effectiveness over the effectiveness of the administration of the HSP complexes or α2M complexes administered alone or the treatment modalities administered alone. In yet another specific embodiment, a first container containing a purified HSP complexes or α2M complexes in an amount ineffective to treat a disease or disorder when administered alone; and a second container and third container, each containing a non-HSP and non-α2M based treatment modality in an amount that, when administered before, concurrently with, or after the administration of the HSP complexes or α2M complexes in the first container, is effective to improve overall treatment effectiveness over the effectiveness of the administration of HSP complexes or α2M complexes administered alone or treatment

modalities administered alone. In a preferred specific embodiment, the invention provides a kit comprising in a first container, a purified HSP complexes or α2M comprising a population of noncovalent HSP-peptide complexes or α2M-peptide complexes of the invention; in a second container, a composition comprising an anti-cancer agent; and in a third container, a composition comprising a cytokine or an adjuvant.

[0296] The kit may for example comprise metal or plastic foil, such as a blister pack. The kit may be accompanied by one or more reusable or disposable device(s) for administration (e.g, syringes, needles, dispensing pens) and/or instructions for administration.

5.8. DETERMINATION OF IMMUNOGENICITY OF THE HSP AND α2M COMPLEXES

[0297] In yet another embodiment, the HSP-peptide complexes and α2M-peptide complexes of the invention can be assayed for immunogenicity using any method known in the art. By way of example but not limitation, one of the following procedures can be used. In a preferred embodiment, the ELISPOT assay is used (see, infra, Section 6.1.7).

[0298] These methods can further be used to compare the immunogenicity of complexes prepared using different sets of antigenic peptides. Such comparisons can reveal the best set of antigenic peptides or combination of sets of antigenic peptides for use as a pharmaceutical composition.

5.8.1. THE MLTC ASSAY

[0299] Briefly, mice are injected with an amount of the HSP- and/or α2M complexes, using any convenient route of administration. As a negative control, other mice are injected with, e.g., HSP complexed to proteins and/or peptides prepared from normal tissue. Cells known to contain specific antigens, e.g. tumor cells or cells infected with an agent of an infectious disease, may act as a positive control for the assay. The mice are injected twice, 7-10 days apart. Ten days after the last immunization, the spleens are removed and the lymphocytes released. The released lymphocytes may be re-stimulated subsequently in vitro by the addition of dead cells that expressed the antigen of interest.

[0300] For example, $8x10^6$ immune spleen cells may be stimulated with $4x10^4$ mitomycin C treated or γ-irradiated (5-10,000 rads) cells containing the antigen of interest (or cells transfected with an appropriate gene, as the case may be) in 3ml RPMI medium containing 10% fetal calf serum. In certain cases 33% secondary mixed lymphocyte culture supernatant may be included in the culture medium as a source of T cell growth factors (See, Glasebrook, et al., 1980, J. Exp. Med. 151:876). To test the primary cytotoxic T cell response after immunization, spleen cells may be cultured without stimulation. In some

experiments spleen cells of the immunized mice may also be re-stimulated with antigenically distinct cells, to determine the specificity of the cytotoxic T cell response.

[0301] Six days later the cultures are tested for cytotoxicity in a 4 hour ^{51}Cr-release assay (See, Palladino, et al., 1987, Cancer Res. 47:5074-5079 and Blachere, at al., 1993, J. Immunotherapy 14:352-356). In this assay, the mixed lymphocyte culture is added to a target cell suspension to give different effector:target (E:T) ratios (usually 1:1 to 40:1). The target cells are prelabelled by incubating $1x10^6$ target cells in culture medium containing 20 mCi ^{51}Cr/ml for one hour at 37°C. The cells are washed three times following labeling. Each assay point (E:T ratio) is performed in triplicate and the appropriate controls incorporated to measure spontaneous ^{51}Cr release (no lymphocytes added to assay) and 100% release (cells lysed with detergent). After incubating the cell mixtures for 4 hours, the cells are pelletted by centrifugation at 200g for 5 minutes. The amount of ^{51}Cr released into the supernatant is measured by a gamma counter. The percent cytotoxicity is measured as cpm in the test sample minus spontaneously released cpm divided by the total detergent released cpm minus spontaneously released cpm.

[0302] In order to block the MHC class I cascade a concentrated hybridoma supernatant derived from K-44 hybridoma cells (an anti-MHC class I hybridoma) is added to the test samples to a final concentration of 12.5%.

5.8.2. CD4+ T-CELL PROLIFERATION ASSAY

[0303] Primary T cells are obtained from spleen, fresh blood, or CSF and purified by centrifugation using FICOLL-PAQUE PLUS (Pharmacia, Upsalla, Sweden) essentially as described by Kruse and Sebald, 1992, EMBO J. 11: 3237-3244. The peripheral blood mononuclear cells are incubated for 7-10 days with a source of target antigen, for example, a lysate of cells expressing an antigenic molecule. Antigen presenting cells may, optionally be added to the culture 24 to 48 hours prior to the assay, in order to process and present the antigen in the lysate. The cells are then harvested by centrifugation, and washed in RPMI 1640 media (GibcoBRL, Gaithersburg, Md.). $5x10^4$ activated T cells/well are in RPMI 1640 media containing 10% fetal bovine serum, 10 mM HEPES, pH 7.5, 2 mM L-glutamine, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulphate in 96 well plates for 72 hrs at 37°C., pulsed with 1 μCi ^{3}H-thymidine (DuPont NEN, Boston, Mass.)/well for 6 hrs, harvested, and radioactivity measured in a TOPCOUNT scintillation counter (Packard Instrument Co., Meriden, Conn.).

5.8.3. ANTIBODY RESPONSE ASSAY

[0304] In a certain embodiment of the invention, the immunogenicity of an HSP- or α2M-complex is determined by measuring antibodies produced in response to the

vaccination with the complex. In one mode of the embodiment, microtitre plates (96-well Immuno Plate II, Nunc) are coated with 50 µl/well of a 0.75 µg/ml solution of a purified, non-HSP- or α2M- complexed form of the proteins/peptides used in the vaccine in PBS at 4°C for 16 hours and at 20°C for 1 hour. The wells are emptied and blocked with 200 µl PBS-T-BSA (PBS containing 0.05% (v/v) TWEEN 20 and 1% (w/v) bovine serum albumin) per well at 20°C for 1 hour, then washed 3 times with PBS-T. Fifty µl/well of plasma or CSF from a vaccinated animal (such as a model mouse or a human patient) is applied at 20°C for 1 hour, and the plates are washed 3 times with PBS-T. The anti-peptide antibody activity is then measured calorimetrically after incubating at 20°C for 1 hour with 50µl/well of sheep anti-mouse or anti-human immunoglobulin, as appropriate, conjugated with horseradish peroxidase (Amersham) diluted 1:1,500 in PBS-T-BSA and (after 3 further PBS-T washes as above) with 50 µl of an o-phenylene diamine (OPD)-H_2O_2 substrate solution. The reaction is stopped with 150 µl of 2M H_2SO_4 after 5 minutes and absorbance is determined in a Kontron SLT-210 photometer (SLT Lab-instr., Zurich, Switzerland) at 492 nm (ref. 620 nm).

5.8.4. CYTOKINE DETECTION ASSAY

[0305] The CD4+ T cell proliferative response to HSP- or α2M-complexes of the invention may be measured by detection and quantitation of the levels of specific cytokines. In one embodiment, for example, intracellular cytokines may be measured using an IFN-γ detection assay to test for immunogenicity of a complex of the invention. In an example of this method, peripheral blood mononuclear cells from a subject treated with a HSP-peptide or α2M peptide complex are stimulated with peptide antigens of a given tumor or with peptide antigens of an agent of infectious disease. Cells are then stained with T cell-specific labeled antibodies detectable by flow cytometry, for example FITC-conjugated anti-CD8 and PerCP-labeled anti-CD4 antibodies. After washing, cells are fixed, permeabilized, and reacted with dye-labeled antibodies reactive with human IFN-γ (PE- anti-IFN-γ). Samples are analyzed by flow cytometry using standard techniques.

[0306] Alternatively, a filter immunoassay, the enzyme-linked immunospot assay (ELISPOT) assay, may be used to detect specific cytokines surrounding a T cell. In one embodiment, for example, a nitrocellulose-backed microtiter plate is coated with a purified cytokine-specific primary antibody, i.e., anti-IFN-γ, and the plate is blocked to avoid background due to nonspecific binding of other proteins. A sample of mononuclear blood cells, containing cytokine-secreting cells, obtained from a subject treated with a HSP-peptide and/or α2M peptide complex, which sample is diluted onto the wells of the microtitre plate. A labeled, e.g., biotin-labeled, secondary anti-cytokine antibody is added.

The antibody cytokine complex can then be detected, i.e. by enzyme-conjugated streptavidin – cytokine-secreting cells will appear as "spots" by visual, microscopic, or electronic detection methods.

5.8.5. TETRAMER ASSAY

[0307] In another embodiment, the "tetramer staining" assay (Altman et al., 1996, Science 274: 94-96) may be used to identify antigen-specific T-cells. For example, in one embodiment, an MHC molecule containing a specific peptide antigen, such as a tumor-specific antigen, is multimerized to make soluble peptide tetramers and labeled, for example, by complexing to streptavidin. The MHC-peptide antigen complex is then mixed with a population of T cells obtained from a subject treated with a HSP- or α2M-complex. Biotin is then used to stain T cells which express the antigen of interest, i.e., the tumor-specific antigen.

5.9. MONITORING OF EFFECTS DURING CANCER PREVENTION AND IMMUNOTHERAPY

[0308] The effect of immunotherapy with HSP- or α2M-complexes on the development and progression of neoplastic diseases can be monitored by any method known to one skilled in the art, including but not limited to measuring: a) delayed hypersensitivity as an assessment of cellular immunity; b) activity of cytolytic T-lymphocytes in vitro; c) levels of tumor specific antigens, e.g., carcinoembryonic (CEA) antigens; d) changes in the morphology of tumors using techniques such as a computed tomographic (CT) scan; and e) changes in levels of putative biomarkers of risk for a particular cancer in individuals at high risk, and f) changes in the morphology of tumors using a sonogram.

[0309] The following subsections describe optional, exemplary procedures.

5.9.1. DELAYED HYPERSENSITIVITY SKIN TEST

[0310] Delayed hypersensitivity skin tests are of great value in the overall immunocompetence and cellular immunity to an antigen. Inability to react to a battery of common skin antigens is termed anergy (Sato, T., et al., 1995, Clin. Immunol. Pathol. 74:35-43).

[0311] Proper technique of skin testing requires that the antigens be stored sterile at 4°C, protected from light and reconstituted shortly before use. A 25- or 27-gauge needle ensures intradermal, rather than subcutaneous, administration of antigen. Twenty-four and 48 hours after intradermal administration of the antigen, the largest dimensions of both erythema and induration are measured with a ruler. Hypoactivity to any given antigen or

group of antigens is confirmed by testing with higher concentrations of antigen or, in ambiguous circumstances, by a repeat test with an intermediate test.

5.9.2. ACTIVITY OF CYTOLYTIC T-LYMPHOCYTES IN VITRO

[0312] $8x10^6$ Peripheral blood derived T lymphocytes isolated by the Ficoll-Hypaque centrifugation gradient technique, are restimulated with $4x10^4$ mitomycin C treated tumor cells in 3ml RPMI medium containing 10% fetal calf serum. In some experiments, 33% secondary mixed lymphocyte culture supernatant or IL-2, is included in the culture medium as a source of T cell growth factors.

[0313] In order to measure the primary response of cytolytic T-lymphocytes after immunization, T cells are cultured without the stimulator tumor cells. In other experiments, T cells are restimulated with antigenically distinct cells. After six days, the cultures are tested for cytotoxicity in a 4 hour ^{51}Cr-release assay. The spontaneous ^{51}Cr-release of the targets should reach a level less than 20%. For the anti-MHC class I blocking activity, a tenfold concentrated supernatant of W6/32 hybridoma is added to the test at a final concentration of 12.5% (Heike M., et al., J. Immunotherapy 15:165-174).

5.9.2. LEVELS OF TUMOR SPECIFIC ANTIGENS

[0314] Although it may not be possible to detect unique tumor antigens on all tumors, many tumors display antigens that distinguish them from normal cells. The monoclonal antibody reagents have permitted the isolation and biochemical characterization of the antigens and have been invaluable diagnostically for distinction of transformed from nontransformed cells and for definition of the cell lineage of transformed cells. The best-characterized human tumor-associated antigens are the oncofetal antigens. These antigens are expressed during embryogenesis, but are absent or very difficult to detect in normal adult tissue. The prototype antigen is carcinoembryonic antigen (CEA), a glycoprotein found on fetal gut an human colon cancer cells, but not on normal adult colon cells. Since CEA is shed from colon carcinoma cells and found in the serum, it was originally thought that the presence of this antigen in the serum could be used to screen patients for colon cancer. However, patients with other tumors, such as pancreatic and breast cancer, also have elevated serum levels of CEA. Therefore, monitoring the fall and rise of CEA levels in cancer patients undergoing therapy has proven useful for predicting tumor progression and responses to treatment.

[0315] Several other oncofetal antigens have been useful for diagnosing and monitoring human tumors, e.g., alpha-fetoprotein, an alpha-globulin normally secreted by fetal liver and yolk sac cells, is found in the serum of patients with liver and germinal cell tumors and can be used as a matter of disease status.

5.9.3. COMPUTED TOMOGRAPHIC (CT) SCAN

[0316] CT remains the choice of techniques for the accurate staging of cancers. CT has proved more sensitive and specific than any other imaging techniques for the detection of metastases.

5.9.4. MEASUREMENT OF PUTATIVE BIOMARKERS

[0317] The levels of a putative biomarker for risk of a specific cancer are measured to monitor the effect of compositions comprising cytosolic and membrane-derived proteins. For example, in individuals at enhanced risk for prostate cancer, serum prostate-specific antigen (PSA) is measured by the procedure described by Brawer, M.K., et al., 1992, J. Urol. 147:841-845, and Catalona, W.J., et al., 1993, JAMA 270:948-958; or in individuals at risk for colorectal cancer CEA is measured as described above in Section 4.5.3; and in individuals at enhanced risk for breast cancer, 16-α-hydroxylation of estradiol is measured by the procedure described by Schneider, J. et al., 1982, Proc. Natl. Acad. Sci. ISA 79:3047-3051. The references cited above are incorporated by reference herein in their entirety.

5.9.5. SONOGRAM

[0318] A sonogram remains another choice of technique for the accurate staging of cancers.

6. EXAMPLE

[0319] The following experiment demonstrates that complexes of HSC70 and an antigenic set of peptides derived from ovalbumin are effective at inducing an immune response against the target antigen in an animal.

6.1. MATERIALS AND METHODS

6.1.1. HSP

[0320] Recombinant human HSC70 (rh-HSC70), a member of the HSP70 family was obtained by the method described in Section 5.3.7.

6.1.2. GENERATATION OF ANTIGENIC SETS OF PEPTIDES FROM OVALBUMIN BY CHEMICAL AND ENZYMATIC CLEAVAGE

[0321] Ovalbumin from chicken egg white (Sigma) was dissolved in 70% formic acid at a concentration of 5 mg/ml prior to chemical cleavage with cyanogen bromide (CNBr). The protein solution was then incubated with 50 fold molar excess of CNBr (CNBr: methionine) in the dark for 20 hours at room temperature. Alternately, for enzymatic cleavage with Staphylococcus aureus peptidase I (V8 protease), ovalbumin was

dissolved in 0.1M of ammonium bicarbonate buffer, pH 7.8, containing 5mM of Dithiothreitol (DTT) at a concentration of 1 mg/ml and incubated with the protease to a final protease to protein ratio of 1:50 (w/w) at 37°C for 3hr. After cleavage by CNBr or V8, peptides of molecular weight less than 10 KDa was collected by ultrafiltration with Amicon Utral centrifugal filter units (Millipore) with a 10KDa molecular weight barrier, followed by a solid-phase extraction process using Sep-pak C18 reverse phase cartridges (Waters). After elution from the C18 cartridge with 95% acetonitrile / 5% Water/ 0.1% trifluoroacetic acid (TFA), the peptides were lyophilized and dissolved in dimethylsulfoxide (DMSO). The concentrations of generated peptide sets were determined by CBQCA protein quantitation kit (Molecular Probes) using instructions with the kit and by amino acid analysis. The identities of the peptides in the antigenic set of peptides were confirmed by MALDI-TOF mass spectrometry.

6.1.3. COMPLEXING PEPTIDES TO rhHSC 70

[0322] Peptides and purified rh-HSC70 (recombinant human HSC70) were mixed at molar ratios and incubated in binding buffer (PBS without calcium and magnesium) at 37°C for 30 min. Control preparations, rh-HSC70 alone and peptides alone were subjected to the same treatment.

6.1.4. ISOLATION OF MOUSE Hsp70 FROM TISSUE

[0323] Livers and kidneys from naïve C57BL/6 mice were used to prepare Hsp70. The resulting HSP70 preparation comprised constitutive HSP73 and inducible HSP72. Purification was performed using an ATP-agarose column, which removed endogenously bound peptides from the heat shock protein. Liver tissue was homogenized in hypotonic buffer, and the homogenate was subjected to centrifugation at 100,000 x g. The supernatant was passed through blue Sepharose to remove albumin, buffer-exchanged and then applied to an ATP-agarose column in Tris-acetate, 20 mM NaCl buffer. HSP70 was eluted with 0.5 M NaCl. The eluted protein was buffer exchanged into 20 mM sodium phosphate, 20 mM NaCl, pH 7.0 and eluted on MonoQ using a 20-600 mM NaCl gradient. The purified HSP70 was stored at -80°C until required.

6.1.5. COMPLEXING OF PEPTIDES TO mHsp70

[0324] Complexes that were prepared consisted of murine heat shock protein 70 (mHsp70) and ovalbumin peptide pools. The peptide pools consisted of an equal mixture of peptides generated by CNBr cleavage or Staphylococcus peptidase I (V8) digestion. The complexes were prepared from defined molar ratios of peptides and heat shock protein. Because of the heterologous nature of the mixture of peptides resulting from thousands of

expressed open reading frames, an arbitrary average molecular weight of 5,000 was used. To produce mHSP70/OVA CNBr and V8 peptide complex, mHSP70 in phosphate buffered saline (PBS) was incubated at 37°C for 30 minutes with the peptide mixture at 1:5:5 molar ratio of mHSP70 to CNBr and V8 peptides (35 ug of CNBr peptides +35 μg of V8 peptides per 100 μg mHSP70 in a 100 μl injection volume). After the incubation, QS-21 was added to the samples at 10 μg per dose.

6.1.6. IMMUNOGENICITY STUDIES

[0325] Six- to 10-week-old C57BL/6 female mice (H-2^b) were purchased from the Jackson Laboratories (Bar, Harbor, ME). Mice were immunized with 1) 100 μg of rh-HSC70 complexed with an indicated amount of OVA CNBr- and/or V8-generated peptide pool, 2) the equivalent amount of the peptide set alone or 3) 100 μg of rh-HSC70 alone. When indicated 10 μg per injection of QS-21 adjuvant was added to the formulations. The samples were administered intradermally (i.d.) once or twice (one week apart) into the right flank of the mice in 100 μl volume. Seven or eight days after the last immunization, spleens were harvested for immunological assays.

6.1.7. ELISPOT ASSAY

[0326] Ninety-six-well ELISPOT plates (Millipore, Bedford, MA) were coated overnight at 4°C with 100 μl per well of 15 μg/ml anti-mouse IFN-γ monoclonal antibody (clone AN18, Mabtech, Mariemont, OH). The antibody was removed; the plates were washed three times with sterile PBS, and blocked for 2 hr with RPMI 1640 containing 10% heat-inactivated FBS. Splenocytes from a single cell suspension were added at 1×10^6 cells/well with a stimulating antigen (5 μg/ml of SIINFEKL peptide, 10 μg/ml of OVA CNBr or V8 peptide set or 5 μg/ml of an irrelevant SSIEFARL peptide), or control with cell media alone, and incubated at 37°C, 5% CO_2 for 40 hours. In anti-CD8 or anti-CD4 antibody blocking experiments, the splenocytes were pre-incubated for 20 min at room temperature with anti-CD8 antibody, anti-CD4 antibody or IgG isotype control antibody (BioExpress, Lebanon, NH) at 10 μg/ml final concentration, then plated as above. After a 40-hour incubation, the cells were removed by washing the plates with PBS/0.05%Tween-20. The plates were then treated with 100 μl/well of 1 μg/ml of biotinylated anti-mouse IFN-γ monoclonal antibody (clone R4-6A2, Mabtech, Mariemont, OH) and incubated at 37°C, 5%CO_2 for 2 hours. The plates were then washed and treated with 100 μl per well of a 1:100 dilution of streptavidin-HRP (Mabtech, Mariemont, OH) and incubated at room temperature for 1 hour. Wells were then washed with PBS-Tween and developed using 3-amino-9-ethylcarbazole (AEC, Sigma) in 0.1 M acetate buffer, containing 0.05% H_2O_2.

Reactions were allowed to proceed for 4 minutes and the reaction was stopped by extensive washing with dH_2O. Plates were allowed to dry and the resulting spots were counted by Zellnet Consulting Inc. (Fort Lee, NJ).

6.1.8. STUDIES ON PROPHYLACTIC EFFICACY

[0327] Six to ten-week-old C57BL/6 female mice (H-2^b) were purchased from Jackson Laboratories (Bar Harbor, ME). Five groups of mice (12 mice per group) were immunized with (1) PBS, (2) 100 μg mHsp70, (3) 100 μg of OVA CNBr and V8-generated peptide pool, (4) 100 μg of mHsp70 complexed with OVA CNBr and V8-generated peptide pool, in a ratio of 1:5:5 respectively or (5) 25 μg of OVA, respectively. 10 μg of QS-21 adjuvant was administered in the same composition for trials 2-5. The samples were administered intradermally (i.d.) into the mice on day 0 and day 6 in 100 μl volume. On day 17, two mice per group were sacrificed for immunogenicity studies. The remaining mice received $2x10^6$ cells of EG7 OVA tumor cells by injection intradermally into the mice. Tumor measurements were made twice a week until completion of the study on day 40.

6.1.9. STUDIES ON THERAPEUTIC EFFICACY

[0328] Six to ten-week-old C57BL/6 female mice (H-2^b) were purchased from Jackson Laboratories (Bar Harbor, ME). On day 0, the mice were injected with $2x10^6$ EG7.OVA tumor cells intradermally into the mice. On day 5, the first tumor measurements were made, and the mice were divided into 5 groups and randomized with an average tumor size of 5 mm diameter per group. Tumor measurements were made twice a week until day 22. On day 6, 9, 13, 16 and 19, the mice in the respective groups received (1) PBS, (2) 100 μg of mHsp70, (3) 100 μg of OVA CNBr and V8-generated peptide pool, (4) 100 μg of mHsp 70 complexed with OVA CNBr and V8-generarated peptide pool in a ratio of 1:5:5 respectively, or (5) 25 μg of OVA, by injection intradermally. Where indicated, 10 μg per injection of QS-21 adjuvant was also administered in the same composition. On day 22, spleens were harvested for ELISPOT assays.

6.2. RESULTS

6.2.1. GENERATION OF ANTIGENIC SETS OF PEPTIDES FROM PURIFIED TARGET ANTIGEN

[0329] Antigenic sets of peptides were generated from purified protein antigen ovalbumin (OVA), by cleavage separately with CNBr or Staphylococcus aureus peptidase I (V8 protease). Yield of peptides produced by CNBr or V8 protease cleavage of OVA protein is shown in Table 2. Mass spectrometry analysis of CNBr cleavage of ovalbumin and V8 protease cleavage of ovalbumin revealed multiple peaks of peptides indicative of cleavage of the target antigen. OVA peptide sets generated by CNBr cleavage were

complexed to HSP70, and the complexes were shown to elicit strong peptide set-specific CD4 and CD8 responses including CD8+ T cell responses to the OVA-derived K^b-restricted SIINFEKL peptide. The responses were further inhanced by inclusion of QS-21 adjuvant in the preparation. In contrast, peptide sets generated by V8 protease digestion and complexed to HSP70 elicited no K^b/SIINFEKL-specific responses. The lack of a SIINFEKL-specific response would be expected, as V8 cleaves peptide bonds on the carboxyl side of aspartic and glutamic acid residues and hence would destroy the SIINFEKL epitope. Mixture of peptide sets generated from CNBr cleavage and V8 protease digestion and immunization with the peptide sets complexed to HSP70 (and QS21) elicited stronger responses specific for the CNBr peptide set, V8 peptide set or K^b/SIINFEKL than the peptide sets generated from CNBr cleavage or V8 protease digestion alone complexed to HSP70.

Table 2. OVA peptide yields after cleavage

Treatment	Concentration (mg/ml)	Total peptides (mg)	Yields (ug peptide/mg ova)
V8	16.16	12.93	86
CNBr	55.75	55.75	230

6.2.2. ANTIGENIC SETS OF PEPTIDES PRODUCE PEPTIDE-SPECIFIC IMMUNE RESPONSE

[0330] OVA CNBr cleavage-generated peptides complexed to rh-HSC70 induce SIINFEKL- and CNBr-cleaved peptide-specific responses. In these experiments, mice were immunized twice (day 0 and day 7) and splenocytes isolated from immunized mice were analyzed on day 14.

[0331] Fig. 1A depicts a graph of immune responses to stimulating antigen in spleen cells from mice immunized with 1) 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant, 2) 100 μg of rh-HSC70 complexed with OVA CNBr-generated peptide set, or 3) 100 μg of rh-HSC70 complexed with OVA CNBr-generated peptide set plus 10 μg per injection of QS-21 adjuvant. Immune cells from mice vaccinated with 100 μg of rh-HSC70 complexed with OVA CNBr-generated peptide set plus 10 μg per injection of QS-21 adjuvant produced a greater immune response against the SIINFEKL peptide, or a CNBr-generated peptide set, than immune cells of mice immunized with 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant or immune cells of mice immunized with 100 μg of rh-HSC70 complexed with OVA CNBr-generated peptide set.

[0332] Fig. 1B demonstrates that immune cells of mice immunized with rh-HSC70 complexed with OVA CNBr-generated peptide set plus 10 μg per injection of QS-21

adjuvant show a strong CD4- and CD8-specific immune response to the CNBr-generated peptide set. Anti-CD4 or anti-CD8 antibody-blocked cells demonstrated a reduced response to the CNBr-generated peptide set relative to an isotype control, indicating that the immune response to the peptide set is generated in part by CD4 and CD8 antibodies.

[0333] A mixture of OVA CNBr- and V8 protease- cleavage-generated peptides complexed to rh-HSC70 induces SIINFEKL- and CNBr /V8 peptide-specific responses. In these experiments, mice were immunized once (day 1) and tissues were analyzed on day 8.

[0334] Fig. 2A depicts a graph of immune responses to stimulating antigen in spleen cells from mice immunized with 1) 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant, 2) 10.71 μg of OVA CNBr- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant, or 3) 100 μg of rh-HSC70 complexed with OVA CNBr-cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant. Immune cells from mice vaccinated with 100 μg of rh-HSC70 complexed with OVA CNBr-generated peptide set plus 10 μg per injection of QS-21 adjuvant produced a greater immune response against the SIINFEKL peptide, or a CNBr-generated peptide set, than immune cells of mice immunized with 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant or immune cells of mice immunized with 10.71 μg of OVA CNBr-generated peptide set alone.

[0335] Fig. 2B depicts a graph of immune responses to antigenic sets of peptides as a stimulating antigen in spleen cells from mice immunized with 1) 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant, 2) 10.71 μg of OVA V8 protease- cleavage-generated peptides plus 10 μg per injection of QS-21 adjuvant, or 3) 100 μg of rh-HSC70 complexed with OVA V8 protease- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant. Immune cells from mice vaccinated with 100 μg of rh-HSC70 complexed with OVA V8 protease- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant elicited no response against the SIINFEKL peptide, and a very low response against V8 protease- cleavage-generated peptide set.

[0336] Fig. 2C depicts a graph of immune responses to stimulating antigen in spleen cells from mice immunized with 1) 100 μg of rh-HSC70 plus 10 μg per injection of QS-21 adjuvant, 2) 21.42 μg of OVA CNBr- and V8 protease- cleavage-generated peptide set plus 10 μg per injection of QS-21 adjuvant, or 3) 100 μg of rh-HSC70 complexed with a mixture of OVA CNBr- and V8 protease- cleavage-generated peptide sets plus 10 μg per injection of QS-21 adjuvant. Immune cells from mice vaccinated with 100 μg of rh-HSC70 complexed with a mixture of OVA CNBr- and V8 protease- cleavage-generated peptide sets plus 10 μg per injection of QS-21 adjuvant produced a greater immune response against the SIINFEKL peptide, or a CNBr-generated peptide set, than immune cells of mice immunized

with 100 µg of rh-HSC70 plus 10 µg per injection of QS-21 adjuvant or immune cells of mice immunized with 24.42 µg of mixture of OVA CNBr- and V8 protease- cleavage-generated peptide sets plus 10 µg per injection of QS-21 adjuvant. Moreover, mixture of antigenic sets of peptides generated from CNBr cleavage and V8 protease digestion and immunization with the peptide sets complexed to HSP70 and administered in combination with QS21 elicited stronger responses specific for the CNBr peptide set, V8 peptide set or K^b/SIINFEKL than the peptide sets generated from CNBr cleavage or V8 protease digestion alone complexed to HSP70.

[0337] Fig. 2D demonstrates that the immune response to CNBr- and V8 protease-cleavage-generated peptides is CD4- and CD8-specific. Graph of immune response to stimulating antigen in spleen cells from mice immunized with 100 µg of rh-HSC70 complexed with OVA CNBr- and V8 protease- cleavage-generated peptide set plus 10 µg per injection of QS-21 adjuvant. Anti-CD4 or anti-CD8 antibody-blocked cells demonstrated a reduced response to the CNBr-generated peptide set and the V8 protease-generated peptide set relative to an isotype control, indicating that the immune response to both CNBr- and V8 protease-generated peptide sets is generated in part by CD4 and CD8 antibodies.

6.2.3. PROPHYLATIC AND THERAPEUTIC STUDIES IN MICE

[0338] Complexes comprising mouse Hsp70 and OVA peptides generated by CNBr and V8 protease induced an immune response that is prophylactically and therapeutically effective in a mosue tumor model.

[0339] In the prophylactic experiments, mice were immunized twice (day 0 and day 7) and tumors measured twice a week until day 40. In the therapeutic experiments, mice were injected with tumor cells on day 0 and injected with 5 treatments on days 6, 9, 13, 16, and 19. Tumor measurements were made twice a week.

[0340] Figure 3A shows the results of the experiment demonstrating a protective effect of the complexes comprising OVA CNBr and V8 generated peptides and HSP 70 in mice when challenged with EG7.OVA tumor cells. Relative to the diluent negative control (PBS), hsp protein negative control (mHsp70 with QS-21), and OVA peptide control (OVA CNBr and V8 peptides with QS-21), the mean tumor volume for the experimental (OVA CNBr and V8 generated peptides complexed to HSP 70 and QS-21) was lower.

[0341] Figure 3B shows the results of the experiment demonstrating a therapeutic effect of the complexes comprising OVA CNBr and V8 generated peptides and HSP 70 in mice when harbored EG7.OVA tumor cells. When compared to the diluent negative control (PBS), hsp protein negative control (mHsp70 with QS-21), and OVA peptide control (OVA

CNBr and V8 peptides with QS-21), the mean tumor volume for the experimental (OVA CNBr and V8 generated peptides complexed to HSP 70 and QS-21) was 34% lower than the PBS control (P=0.06). The immunization positive control (OVA protein) showed a 73% decrease in mean tumor volume relative to the PBS control (P=0.001).

6.3. CONCLUSION

[0342] HSC70-antigenic peptide complexes elicited T cell responses specific for the CNBr peptide set and V8 peptide set, including the epitope comprising the amino acid sequence SIINFEKL. The immune response is enhanced when the complexes are used in combination with an adjuvant, in this example, a saponon, QS-21. The immune response is further enhanced when peptides from different antigenic sets of peptides are used in making the HSP-antigenic peptide complexes. Therefore, the example demonstrates testing of the immunogenicities of different antigenic sets of peptides obtained by using different methods to fragment a target antigen. The example also demonstrate the combination of different antigenic sets of peptides in the making of HSP complexes which yielded highly immunogenic HSP-antigenic peptide complexes.

[0343] The prophylactic and therapeutic studies were carried out using complexes containing heat shock protein purified from the tissue of a non-transgenic animal. This was done in part to avoid an immune response in mice to non-self HSC70 which were used in the immunogenicity studies. Complexes of mouse Hsp70 and the ovalbumin peptide sets effectively protected the mice from a challenge by tumor inoculation, and reduced the growth in size of a pre-existing tumor. The animal studies clearly show the efficacies of the complexes in preventing and treating a cancer.

[0344] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[0345] Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims along with the full scope of equivalents to which such claims are entitled.

WHAT IS CLAIMED IS:

1. A method of making an immunogenic population of complexes comprising

(i) treating a purified target antigen preparation or combination of different purified target antigen preparations, each said purified target antigen preparation comprising a purified polypeptide target antigen which is a tumor antigen, with a first protease and/or a first non-enzymatic compound that cleaves said polypeptide target antigen to generate a first antigenic set of peptides comprising a plurality of different peptides; and

(ii) complexing said first antigenic set of peptides to a heat shock protein or alpha-2-macroglobulin to form said population of complexes.

2. A method of making an immunogenic population of complexes comprising

(i) treating a purified target antigen preparation or combination of different purified target antigen preparations, each said purified target antigen preparation comprising a purified polypeptide target antigen, with a first protease and/or a first non-enzymatic compound that cleaves said polypeptide target antigen to generate a first antigenic set of peptides comprising a plurality of different peptides; and

(ii) complexing said first antigenic set of peptides to an alpha-2-macroglobulin to form said population of complexes.

3. A method of eliciting an immune response in a subject to one or more target antigens, said method comprising

(i) treating a purified target antigen preparation or combination of different purified target antigen preparations, each said purified target antigen preparation comprising a purified polypeptide target antigen, with a first protease and/or a first non-enzymatic compound that cleaves said polypeptide target antigen to generate a first antigenic set of peptides comprising a plurality of different peptides;

(ii) complexing said first antigenic set of peptides to a heat shock protein or alpha-2-macroglobulin to form a population of complexes comprising (a) heat shock protein or alpha-2-macroglobulin, and (b) peptides of said one or more target antigens; and

(iii) administering to said subject a composition comprising said population of complexes.

4. A method of treating or preventing a type of cancer in a subject, said method comprising

(i) treating a purified target antigen preparation or combination of different purified target antigen preparations, each said purified target antigen preparation comprising a purified polypeptide target antigen, with a first protease and/or a first non-enzymatic

compound that cleaves said polypeptide target protein to generate a first antigenic set of peptides; wherein each said purified polypeptide target antigen is a tumor antigen of said type of cancer;

(ii) complexing said first antigenic set of peptides to a heat shock protein or alpha-2-macroglobulin to form a population of complexes comprising (a) a heat shock protein or alpha-2-macroglobulin, and (b) peptides of said target antigen; and

(iii) administering to said subject a composition comprising said population of complexes.

5. A method of treating or preventing a type of infectious disease, comprising

(i) treating a purified target antigen preparation or combination of different purified target antigen preparations, each said purified target antigen preparation comprising a purified polypeptide target antigen, with a first protease and/or a first non-enzymatic compound that cleaves said polypeptide target antigen to generate a first antigenic set of peptides; wherein said target antigen is an antigen associated with said type of infectious disease;

(ii) complexing said first antigenic set of peptides to a heat shock protein or alpha-2-macroglobulin to form a population of complexes comprising (a) a heat shock protein or alpha-2-macroglobulin, and (b) peptides of said target antigen; and

(iii) administering to said subject a composition comprising said population of complexes.

6. The method of claim 1 or 3, further comprising, before step (ii), treating said first antigenic set of peptides with a second protease and/or a second non-enzymatic compound that cleaves said polypeptide target antigen.

7. The method of any of claims 1 to 5, further comprising, treating said purified target antigen preparation separately with a second protease and/or a second non-enzymatic compound that cleaves said polypeptide target antigen to form a second antigenic set of peptides, and combining said first and second antigenic sets of peptides before complexing to the heat shock protein or alpha-2-macroglobulin in step (ii).

8. The method of any of claims 1 to 5, further comprising, treating said purified target antigen preparation separately with a second protease and/or a second non-enzymatic compound that cleaves said polypeptide target antigen to form a second antigenic set of peptides, complexing said second antigenic sets of peptides separately to the heat shock

protein or alpha-2-macroglobulin to form a population of complexes which is mixed with the population of complexes of step (ii).

9. The method of any of claims 1 to 5, wherein said first protease is trypsin, staphylococcal peptidase I, chymotrypsin, pepsin, cathepsin G, thermolysin, elastase, peptidylglutamylpeptide-hydrolase, a caspase, or papain.

10. The method of any of claims 1 to 5, wherein said first non-enzymatic compound is cyanogen bromide (CNBr), hydroxylamine, or iodosobenzoic acid.

11. The method of claim 6, wherein said second protease is trypsin, staphylococcal peptidase I, chymotrypsin, pepsin, cathepsin G, thermolysin, elastase, peptidylglutamylpeptide-hydrolase, a caspase, or papain.

12. The method of claim 6, wherein said second non-enzymatic compound is cyanogen bromide (CNBr), hydroxylamine, or iodosobenzoic acid.

13. The method of claim 3, 4, or 5 said method further comprises administering to said subject one or more adjuvants.

14. The method of claim 3, 4, or 5, wherein said one or more adjuvant is QS -21.

15. The method of claim 4, wherein said method further comprises administering to said subject a chemotherapeutic agent, an anti-angiogenic agent, a cytokine, a biological response modifier, a hormone, an antibody, a polynucleotide, an immunostimulatory oligonucleotide, a photodynamic therapeutic agent or radiation.

16. The method of claim 5, wherein said method further comprises administering to said subject an antibiotic, an antiviral compound, an antiprotozoal compound, an antifungal compound, an antihelminthic compound, an antibody, a cytokine, a hormone, an immunostimulatory oligonucleotide, a biological response modifier, or a polynucleotide.

17. The method of claim 3 or 4, wherein said target antigen is a unique tumor antigen, a shared tumor specific antigen, a shared tumor associated antigen, a differentiation antigen, or an antigen overexpressed in a tumor.

18. The method of claim 1 or 3, wherein said target antigen is MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-5, MAGE-6, MAGE-7, MAGE-8, MAGE-9, MAGE-10, MAGE-11, MAGE-12, BAGE-1, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5,

GAGE-6, GAGE-7, GAGE-8, LAGE-1, LAGE-2, SSX-2, tyrosinase, carcinoembryonic antigen (CEA), gp100/Pmel17, kallikrein 4, mammaglobin A, Melan-A, TRP-1, TRP-2, prostate specific antigen (PSA), Her-2/neu, CPSF, EphA3, alphafetoprotein, WT-1, telomerase, MUC-1, p53, PRAME, RAGE-1, or PSMA.

19. The method of claim 3 or 5, wherein said target antigen is an antigen of herpes simplex virus type II.

20. The method of claim 19, wherein said target antigen is VP16, VP26, RS1, UL11, UL13, UL18, UL33, UL36, UL37, UL40, UL41, UL45, UL46, UL49, UL54, US9, US11, RL2, , RS1, UL1, UL10, UL14, UL16, UL17, UL20, UL22, UL27, UL34, UL36, UL37, UL44, UL48, UL53, US5, US6, US9, US10, US11, gB, gC, gD, gH, gJ, gK, gL, or gM.

21. The method of claim 3, 4, or 5 wherein said subject is a human being.

22. The method of claim 1 or 3, wherein said population of complexes is purified.

23. The method of claim 4 or 5, wherein said composition is administered to said subject for treatment.

24. The method of claim 4 or 5, wherein said composition is administered to said subject for prevention.

25. The method of claim 1, 3, 4, 5, or 23 wherein said heat shock protein is HSP 60, HSP 70, HSC 70, HSP 90, gp96, calreticulin, grp78, BiP, protein disulfide isomerase, HSP110, or grp170, a combination of two or more thereof.

26. A composition comprising an immunogenic population of complexes comprising (a) heat shock protein or alpha-2-macroglobulin and (b) a plurality of different peptides, said complexes prepared by a method comprising:

(i) treating a purified target antigen preparation or combination of different purified target antigen preparations, each said purified target antigen preparation comprising a purified polypeptide target antigen which is a tumor antigen, with one or more proteolytic composition comprising a protease and/or a non-enzymatic compound that cleaves said polypeptide target antigen to generate a antigenic set of peptides comprising a plurality of different peptides; and

(ii) complexing said antigenic set of peptides to heat shock protein or alpha-2-macroglobulin to form said population of complexes.

27. A composition comprising an immunogenic population of complexes comprising alpha-2-macroglobulin and a plurality of different peptides, said complexes prepared by a method comprising:

(i) treating a purified target antigen preparation or combination of different purified target antigen preparations, each said purified target antigen preparation comprising a purified polypeptide target antigen, with one or more proteolytic composition comprising a protease and/or a non-enzymatic compound that cleaves said polypeptide target antigen to generate a antigenic set of peptides comprising a plurality of different peptides; and

(ii) complexing said antigenic set of peptides to alpha-2-macroglobulin to form said population of complexes.

28. Use of an immunogenic population comprising complexes of a heat shock protein or alpha-2-macroglobulin with peptides of one or more purified target antigens in the manufacture of a medicament for eliciting a Th-1 type immune response in a subject, said peptides being produced by proteolytic digestion of said one or more purified target antigens.

29. The use of claim 28 wherein the purified target antigen is present in a cell when infected with an infectious agent that causes said type of infectious disease, is in an infectious agent that causes said type of infectious disease, or comprises an antigenic determinant of an infectious agent that causes said type of infectious disease

30. The use fo claim 28, wherein the medicament is useful for treating or preventing an infectious disease in the subject.

31. The use of claim 28 wherein the purified target antigen is a unique tumor antigen, a shared tumor specific antigen, a shared tumor associated antigen, a differentiation antigen, or an antigen overexpressed in cells or tissue of said type of cancer.

32. The use fo claim 28, wherein the medicament is useful for treating or preventing cancer in a patient.

33. The use of claim 28, wherein the complexes are prepared by treating said one or more purified polypeptide target antigens with a proteolytic composition comprising a

protease or a non-enzymatic compound which cleaves said polypeptide target antigen to form the proteolytic peptide digests and complexing said digests to heat shock protein or alpha-2-macroglobulin.

34. The method of claim 3, where said immune response is a cell-mediated immune response, a humoral immune response, a cytotoxic T cell response, a NK cell response, or an eosinophil cell response.

35. The method of claim 4, wherein said tumor antigen is a unique tumor antigen, a shared tumor specific antigen, a shared tumor associated antigen, a differentiation antigen, or an antigen overexpressed in cells or tissue of said type of cancer.

36. The method of claim 5, where said target antigen is an antigen that is present in a cell when infected with an infectious agent that causes said type of infectious disease, that is present in an infectious agent that causes said type of infectious disease, or that comprises an antigenic determinant of an infectious agent that causes said type of infectious disease.

37. The method of claim 3 or 5, wherein said target antigen is an antigen of a virus, an antigen of a bacterium, an antigen of a protozoan, an antigen of a fungus, or an antigen of a parasite.

38. The method of claim 3 or 4, wherein said target antigen is an antigen of a renal cell carcinoma, melanoma, lung cancer, breast cancer, lymphoma, leukemia, colorectal cancer or pancreatic cancer.

39. The method of claim 3 or 5, wherein said target antigen is an antigen of a hepatitis virus, human immunodeficiency virus, human papilloma virus, a herpes viruses; Mycobacterium tuberculosis, an influenza virus, Bacillus anthracis, Staphylococcus aureus, Heliobacter pylori, a species of Streptococcus, Plasmodium falciparum, or a Leishmania parasite.The method of any of claims 1 to 5, wherein said heat shock protein or alpha-2-macroglobulin is a human heat shock protein or a human alpha-2-macroglobulin.

40. The method of any of claims 3 to 5, wherein said administeration is intradermal or subcutaneous.A composition of claim 25 or 26, wherein said composition further comprises one or more adjuvants, and/or at least one cytokine selected from the group consisting of an interferon, an interleukin, a colony stimulating factor, and a tumor necrosis factor.

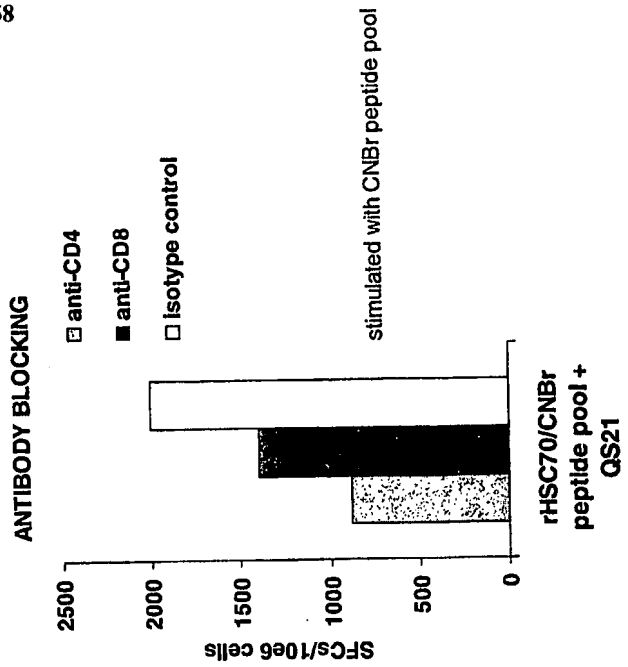

FIGURE 1B

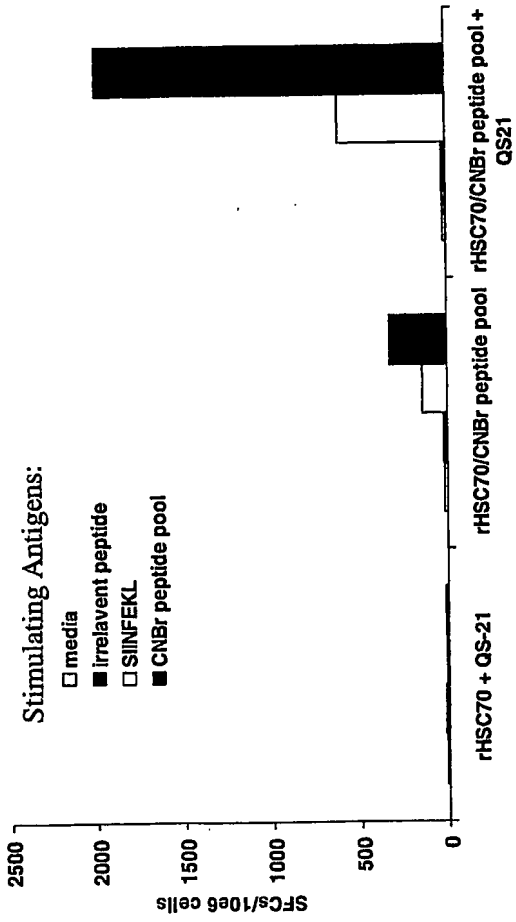

FIGURE 1A

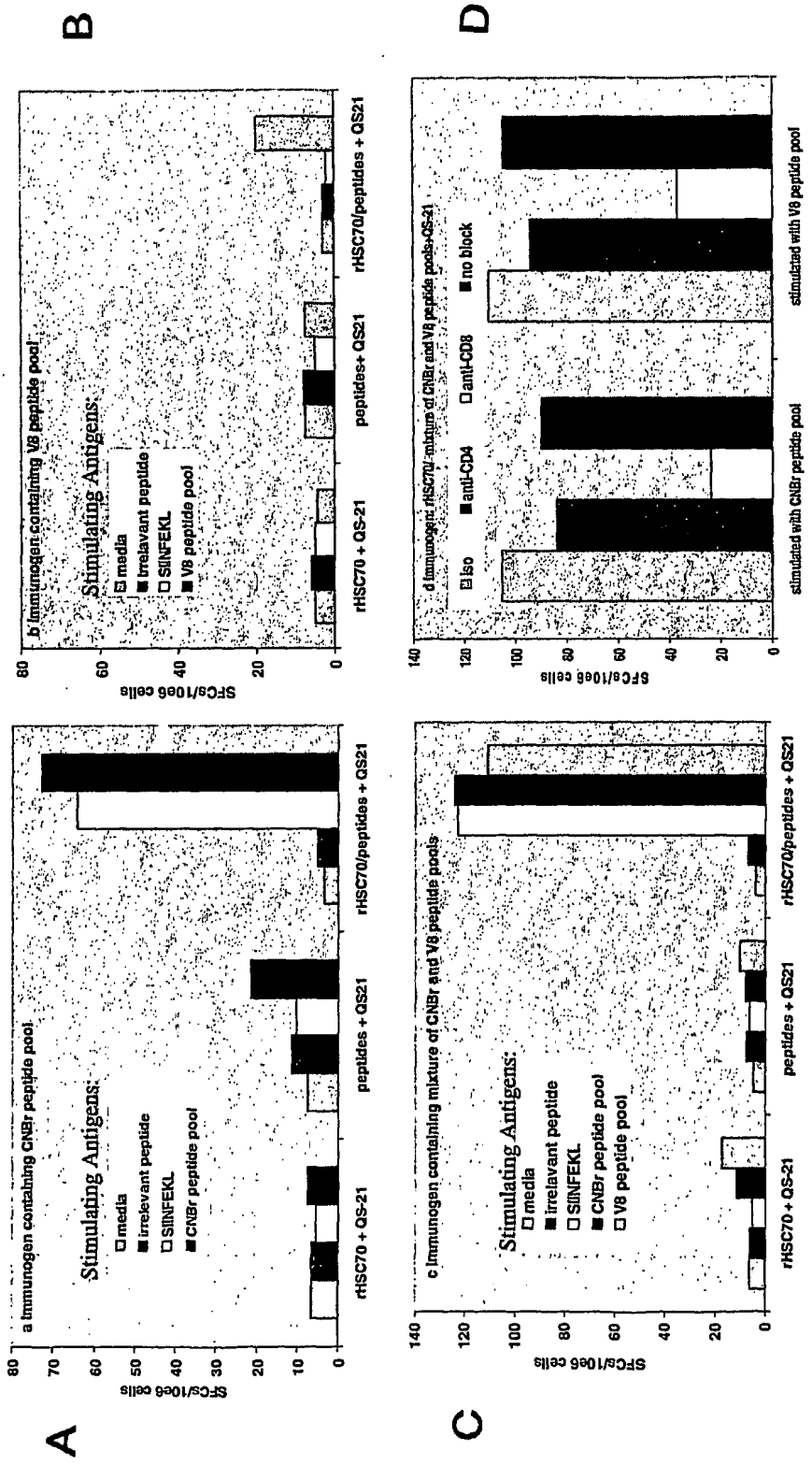
A
a Immunogen containing CNBr peptide pool
Stimulating Antigens:
media
irrelevant peptide
SIINFEKL
CNBr peptide pool
SFCs/10e6 cells
rHSC70 + QS-21
peptides + QS21
rHSC70/peptides + QS21
B
b Immunogen containing V8 peptide pool
Stimulating Antigens:
media
irrelevant peptide
SIINFEKL
V8 peptide pool
SFCs/10e6 cells
rHSC70 + QS-21
peptides+ QS21
rHSC70/peptides + QS21
C
c Immunogen containing mixture of CNBr and V8 peptide pools
Stimulating Antigens:
media
irrelevant peptide
SIINFEKL
CNBr peptide pool
V8 peptide pool
SFCs/10e6 cells
rHSC70 + QS-21
peptides + QS21
rHSC70/peptides + QS21
D
d Immunogen: rHSC70 - mixture of CNBr and V8 peptide pools+QS-21
Iso
anti-CD4
anti-CD8
no block
SFCs/10e6 cells
stimulated with CNBr peptide pool
stimulated with V8 peptide pool

FIGURE 2

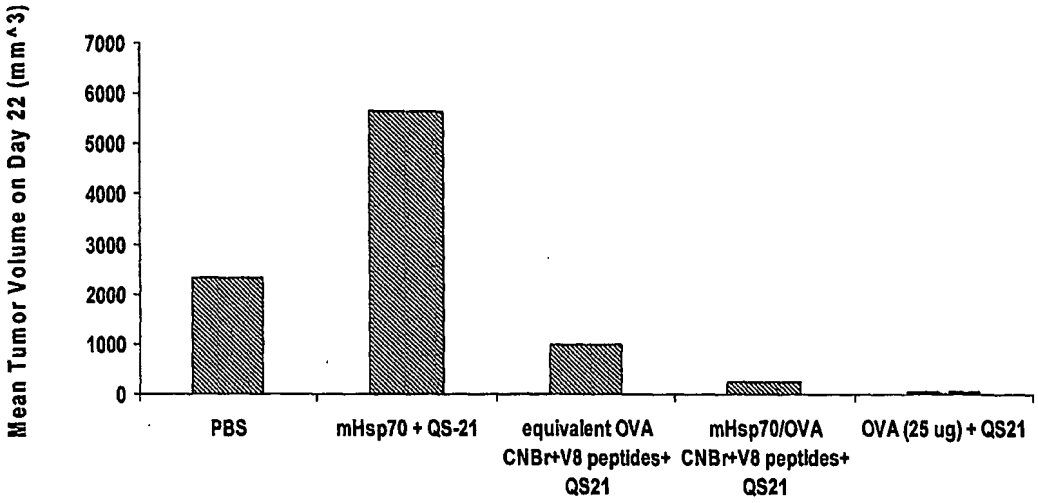

FIGURE 3A

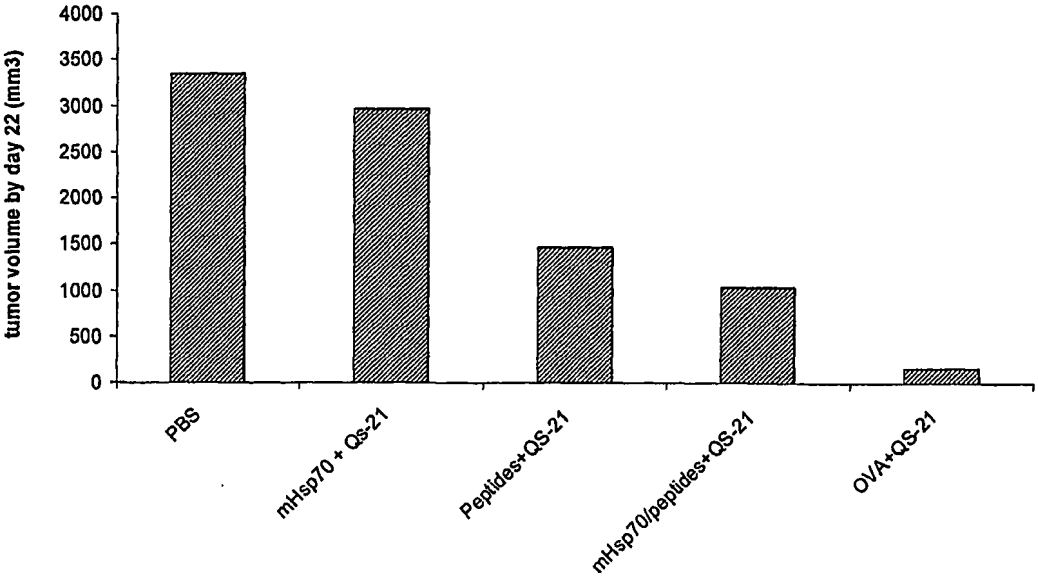

FIGURE 3B